Efecto del genotipo del árbol huésped y de la recurrencia de incendios sobre las comunidades de hongos en pinares Mediterráneos

Effect of the host tree genotype and the fire recurrence on fungal communities in Mediterranean pine forests

TESIS DOCTORAL

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A mis padres,

A Koldo

"No hay inversión más rentable que la del conocimiento"
(Benjamin Franklin)

"Sigan ustedes sabiendo que, mucho más temprano que tarde, se abrirán las grandes alamedas por donde pase el hombre libre para construir una sociedad mejor"
(Salvador Allende)

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ABSTRACT

Many ecological studies have been focused on aboveground plant communities, while much less attention has been paid to belowground microbial communities. However, microorganisms represent a significant fraction of forest ecosystems being directly involved in the cycling of nutrients and the productivity of trees, and elucidating their interactive and intricate relationships with the surrounding environment constitutes one of the major challenges in fungal ecology at present.

The main objective of this thesis was to study the structural and functional responses of belowground fungal communities to principal biotic and abiotic factors (i.e., tree species identity and/or genotype, seasonality, edaphic compartment, fire recurrence) in Mediterranean pine forests ecosystems, and to explore possible mechanisms governing these plant-fungal-environment feedbacks.

In order to achieve these objectives, soil properties and microbial communities in two edaphic compartments (i.e., root-tips and bulk soil) were studied by different methodological approaches combining metagenomics (454 GS-FLX pyrosequencing, Miseq) and phylogenetic techniques, with enzymatic and spectral infrared (NIRs/MIRs) soil analyses. Three independent experimental designs were used to carry out the study.

We showed that the tree species identity and genotype, together with spatial-temporal variations and ecosystem development (fire regime), were key agents determining the edaphic processes as well as shaping the structure and functional responses of fungal and bacterial communities in Mediterranean pine forests. Differential responses were reported for ecological and phylogenetic distinct fungal guilds, and concrete ecosystem processes were identified associated with specific phylogenetic clades. Particularly, we observed that high productive *P. pinaster* genotypes generated different soil quality and associated different ectomycorrhizal assemblages where Basidiomycetes prevailed, while less productive trees preferentially associated Ascomycetes. Likewise, a high fire recurrence drove the phylogenetic clustering of soil fungal communities in *P. halepensis* forests, where Basidiomycetes were over-represented. These structural adjustments entailed functional consequences on nutrient cycling processes. *Pinus pinaster* and *P. halepensis*, which harboured different fungal species assemblages, diverged in their functional response to the fire regime. In addition, spatial partitioning and niche differentiation processes were evidenced between rhizospheric and bulk soil interfaces. Phylogenetic approaches emerged as an important tool to study the relations among microbial diversity and ecosystem functioning. Furthermore, we developed a new molecular marker that can be used in environmental metagenomics studies to link community composition to fungal functions.

Our results provide a holistic view of the plant-microbial-environment interactions, giving evidences for understanding and predicting the main variables structuring fungal communities in Mediterranean pine forests, as well as the potential effects of structural shifts on relevant ecosystem processes. Deciphering the role and underlying mechanisms of plant-soil-microbial feedbacks in driving diversity and functional patterns at the ecosystem level, will allow us making predictions to tackle future climate change scenarios in Mediterranean forests, and to foster the sustainable management of these especially vulnerable ecosystems.

RESUMEN

La mayoría de estudios en ecología se han centrado en las comunidades vegetales, mientras que las comunidades microbianas del suelo han recibido mucha menos atención. Sin embargo, los microorganismos son un componente esencial de los ecosistemas forestales ya que están directamente involucrados en los ciclos de nutrientes y en la productividad de los árboles. Así pues, dilucidar sus relaciones con el entorno ambiental constituye actualmente uno de los mayores retos en ecología de comunidades fúngicas.

El objetivo principal de esta tesis fue estudiar las respuestas estructurales y funcionales de las comunidades fúngicas a importantes factores bióticos y abióticos (identidad y/o genotipo de la especie arbórea, estacionalidad, compartimiento edáfico, recurrencia de incendios) en ecosistemas forestales de pinos Mediterráneos, así como explorar los posibles mecanismos que regulan estas interacciones planta-hongo-ambiente.

Para lograr estos objetivos, se estudiaron las propiedades del suelo y las comunidades microbianas en dos compartimentos edáficos (raíces cortas y matriz del suelo) mediante diferentes enfoques metodológicos combinando metagenómica (454 GS-FLX pirosecuenciación, Miseq) y técnicas filogenéticas, así como determinaciones enzimáticas y espectroscopia del infrarrojo (NIRs / MIRs) en suelos. Para llevar a cabo el estudio, se utilizaron tres diseños experimentales independientes.

Nuestros resultados demostraron que la identidad y el genotipo de las especies arbóreas, junto con las variaciones espacio-temporales y el régimen de incendios, fueron factores clave para determinar los procesos edáficos y modular las respuestas estructurales y funcionales de las comunidades fúngicas y bacterianas en los bosques de pino Mediterráneo. Se observaron diferentes respuestas de consorcios de hongos ecológica y filogenéticamente distintos, y se identificaron procesos ecosistémicos concretos asociados a clados filogenéticos específicos. En particular, se observó que el genotipo Mediterraneo de *Pinus pinaster*, más productivo, generaba diferente calidad de suelo y asociaba diferentes ensamblajes de hongos ectomicorrícicos, con prevalencia de Basidiomicetes, mientras que los árboles del genotipo Atlántico, menos productivos, mostraron mayor preferencia por Ascomicetes. Asimismo, la alta recurrencia de incendios provocó el agrupamiento filogenético de las comunidades fúngicas del suelo en bosques de *Pinus halepensis*, donde los Basidiomicetes estaban sobrerrepresentados. Estos ajustes estructurales implicaron consecuencias funcionales en los procesos relacionados con el ciclado de nutrientes. Los distintos ensamblajes fúngicos de *Pinus pinaster* y *P. halepensis* divergieron en su respuesta funcional al régimen de incendios. Además, se puso en evidencia la separación espacial y la diferenciación de nicho entre la rizosfera y la matriz del suelo. El enfoque filogenético emerge como herramienta importante para estudiar las relaciones entre la diversidad microbiana y el funcionamiento de los ecosistemas. Al mismo tiempo, hemos desarrollado un nuevo marcador

molecular potencialmente utilizable en estudios de metagenómica con muestras ambientales con el objetivo de vincular composición de la comunidad fúngica con su funcionalidad.

Nuestros resultados proporcionan una visión holística de las interrelaciones planta-microorganismos-ambiente, dando evidencias para entender y predecir las principales variables que estructuran las comunidades de hongos en los bosques de pino Mediterráneo, así como los efectos potenciales de esos cambios estructurales en relevantes procesos ecosistémicos. Comprender el papel de las interrelaciones planta-suelo-microorganismos y los mecanismos subyacentes que determinan la diversidad y los patrones funcionales a nivel de ecosistema, nos permitirá hacer predicciones para abordar futuros escenarios de cambio climático en bosques Mediterráneos y fomentar el manejo sostenible de estos ecosistemas especialmente vulnerables.

GENERAL INTRODUCTION

INTRODUCTION

Trees are responsible for a large part of the total primary production in forests ecosystems, where they play pivotal roles in the aboveground/belowground interactions acting as ecosystem engineers (Ellison *et al.*, 2005). The growth of trees in forests soils over the long term allows the creation of a wide and dynamic set of microbial habitats in which soil horizons, rhizosphere, leaf litter and decaying wood/roots can be considered as reactive compartments where most of the nutrients are cycled and microorganisms are specialized (Uroz *et al.*, 2016). Within this framework, fungi and bacteria are key players in carbon and nitrogen cycling and are responsible for ca. 90% of all organic matter decomposition providing plants with essential nutrients (van der Heijden *et al.*, 2008; McGuire and Treseder, 2010).

Many ecological studies have focused on aboveground plant communities, while much less importance has been given to belowground microbial communities. Because soil fungi represent a significant fraction of forest ecosystems that are directly involved in the biogeochemical cycling of nutrients and the productivity of trees (Smith and Read, 2008), there is an increasing interest in elucidating their interactive and intricate relationships with the surrounding environment, which is indeed one of the major challenges in fungal ecology at present (van der Heijden *et al.*, 2015). In this thesis, we approach this topical subject by studying the effect of biotic (i.e., tree genotype and species identity) and abiotic (i.e., season, site) factors, as well as environmental disturbances (i.e., fire recurrence) on forest microbial communities, and questioning whether changes in their structure may trigger functional responses affecting fundamental ecosystem services. Deciphering the role and underlying mechanisms of plant-soil-microbial feedbacks in driving diversity and functional patterns at the ecosystem level, will allow us making predictions to tackle future climate change scenarios in Mediterranean forests, and to foster the sustainable management of these vulnerable ecosystems.

Microbial communities in forests ecosystems

The soil microbiota is fundamental for the ecosystem functioning and significantly influences the diversity and structure of aboveground communities (Kardol and Wardle, 2010). Microbial soil communities create the trophic base for detrital foodwebs, drive global carbon and nutrient cycles, and improve soil structure (Sinsabaugh and Follstad Shah, 2012). Root-soil interfaces constitute the rhizosphere, an active microcosm where plant roots, microorganisms, and soil components interact (Lynch and de Leij, 2001). The rhizosphere is a highly diverse environment where mycorrhizal fungi and bacteria predominate, influencing directly the plant fitness and the soil quality (Barea *et al.*, 2002) .The “rhizhosphere effect” is mainly maintained by root exudates, and modifies the physicochemical characteristics of the surrounding environment

(Hartmann *et al.*, 2009), influencing the structure and metabolism of fungal and bacterial communities (Hartmann *et al.*, 2009).

Among microbial communities, fungi play key ecological roles mainly as decomposers, mutualists, endophites and pathogens (Tedersoo and Smith, 2013), and they constitute most of the microbial biomass in forest soils (Markkola *et al.*, 1995; Wallander *et al.*, 2001; Baldrian, Větrovský, *et al.*, 2013). The diversity of fungi is estimated in 1.5 million of species, of which only around 100 000 taxa have been described (Hibbett *et al.*, 2011).

Saprotrophic fungi are the principal decomposers of wood and litter (Baldrian *et al.*, 2011). They dominate the surface of the forest floor where most C is mineralized (Lindahl *et al.*, 2007; Sinsabaugh *et al.*, 2008; Voříšková *et al.*, 2014). These fungi actively decompose the organic matter through excretion of a potent and diverse array of enzymes including proteinases, cellulases, and laccases (Baldrian and Valášková, 2008). It is assumed that saprotrophic fungi are the main group responsible for litter decomposition in forests. However, mutualist ectomycorrhizal fungi retain an extensive decay genetic machinery enabling them to break down nutrient- and C-rich molecules present in soil and litter, and may also play central roles in C and N dynamics in forests (Talbot *et al.*, 2008; Courty *et al.*, 2010; Kohler *et al.*, 2015; Lindahl and Tunlid, 2015).

The ectomycorrhizal symbiosis

The mycorrhizal symbiosis is the mutualistic relationship between fungi and plants, where basically the fungi supply water and nutrients to plants in exchange for photo-assimilated carbohydrates (Smith and Read, 2008). Up to seven types of mycorrhizas have been described based on their structural and functional features, and the identity of the plants and fungi involved (Smith and Read, 2008). The most representative types are the arbuscular mycorrhizas (AM) and the ectomycorrhizas (ECM). It is estimated that 74% of the plant species are able to form AMs, while only 2% form ECMs (Brundrett, 2009). Oppositely, a reduced number of fungi are involved in the AM symbiosis (i.e., Glomeromycota), while ca. 25000 taxa, belonging to more than 60 independently evolved lineages, are known to form ECM and still much diversity is thought to remain cryptic (Tedersoo *et al.*, 2012; Tedersoo and Smith, 2013). Despite the relatively small number of ECM plants, they have a great global economical and ecological importance because they represent the forest ecosystems. Many tree species such as those in Betulaceae, Pinaceae, Fagaceae, and Dipterocaraceae families are important and obligate ECM plants (Smith and Read, 2008). Most ECM fungi have a broad host range, although some are more specific and colonize only certain host genera or species (Molina *et al.*, 1992).

The ECM symbiosis has evolved multiple times both in plants and fungi (Bittleston *et al.*, 2016). Most ECM fungi belong to the phyla Basidiomycota and Ascomycota (Tedersoo *et al.*,

2010). Despite their independent origins, the morphology of ectomycorrhizas is convergent across lineages and is defined by three structural components: (i) a fungal sheath or mantle around the root, (ii) a network of hyphae (the Hartig net) between the epidermal and cortical cells of the root, and (iii) a mycelium extending from the root into the surrounding soil (Smith and Read, 2008). Through these structures, ECM fungi have direct and advantaged access to root carbohydrates, which makes them highly competitive in the exploration of nutrients in soil (Nehls *et al.*, 2010). In fact, trees can invest more than 30% of the carbon fixed to maintain their associated ECM fungi, which are a great extension of their root system foraging for limiting nutrients in soil, especially nitrogen and phosphorous (Cairney and Burke, 1996; Read and Perez-Moreno, 2003; Smith and Read, 2008). It is estimated that up to 80% of plant N and P can be provided by ECM fungi (van der Heijden *et al.*, 2015). Because the tree generates a flux of carbohydrates towards the roots to maintain the symbiosis, it creates an extremely rich environment where numerous microorganisms, such as bacteria, proliferate (Rincón *et al.*, 2005; Frey-Klett *et al.*, 2007).

Ecology of microbial communities

Given the heterogeneous spatial-temporal distribution patterns of microbial communities, the influence of the edaphic-climatic characteristics, and that of the plant community composition and/or the tree host, understanding the processes that are driving variations of natural bacterial and fungal communities is a major challenge for ecologists (van der Heijden *et al.*, 2015).

In forests, the genotypic and phenotypic characteristics of dominant trees in a site may determine the belowground microbial communities through microclimatic variations and litter chemistry with a strong impact on nutrient dynamics (Treseder and Vitousek, 2001; Madritch and Hunter, 2002). Within this context, mutualistic relationships may represent important mechanisms of plant adaptation to their environment (Johnson *et al.*, 2010). Likewise, temporal-scale variations in fungal and bacterial communities have been linked to the environmental conditions and the phenology of trees (Buée *et al.*, 2005; Taylor *et al.*, 2010; Baldrian, Šnajdr, *et al.*, 2013; Koranda *et al.*, 2013; Voříšková *et al.*, 2014), being the light, soil pH, temperature and moisture (Cooke *et al.*, 1993; Rousk *et al.*, 2010; Coince *et al.*, 2014; Rincón *et al.*, 2015) among the most important abiotic drivers. It is accepted that an interplay of multitude biotic and abiotic factors influence the plant-microbial feedbacks (Peay *et al.*, 2010; Eusemann *et al.*, 2016), although more studies are needed to decipher these complex interrelations.

From a functional point of view, there is an increasing recognition that evolutionary relationships might be important in shaping the functional ecology of plant and microbial communities (Maherali and Klironomos, 2007; Srivastava *et al.*, 2012; Navarro-Cano *et al.*, 2014; Amend *et al.*, 2016). In fact, phylogeny may capture the integrated phenotypic differences among taxa since trait similarity is usually determined by common ancestry (Goberna and Verdú, 2016).

Given the difficulty to quantify functional traits of each and every species in a community, particularly those highly diverse such as microorganisms, considering the functional phylogenetic context is a promising approach to understand the ecology of these communities and to predict the relationship between the structure of ecological communities and the ecosystem functioning (Bruggeman *et al.*, 2009; Pérez-Valera *et al.*, 2015; Talbot *et al.*, 2015; Treseder and Lennon, 2015; Goberna and Verdú, 2016).

Mediterranean ecosystems: adaptation capacity and vulnerability to climate change

The Mediterranean climate is characterized by a marked seasonality with soft winters and hot dry summers, as well as a large year-to-year variability of total rainfalls (Keeley *et al.*, 2011). These conditions make the Mediterranean landscapes some of the most fire-prone ecosystems in the world (Pausas, 2004). Typical characteristics of the Mediterranean region include high geographical and topographical variability, high biodiversity linked to a rich variability of vegetation types and land-use forms resulting in complex mosaics of patches, and a long history of manipulation of trees, forests and landscapes (Scarascia-Mugnozza *et al.*, 2000). Due to the prolonged winter-spring growing season, primary productivity is moderately high for semi-arid regions forming dense vegetation that will lose the moisture in summer becoming very flammable and contributing to fire spread during the dry season (Keeley *et al.*, 2011). These harsh ecological conditions boost the adaptation of the Mediterranean vegetation, which usually shows morphological, phenological, and physiological adjustments (Tapias *et al.*, 2004). For example, among the mechanisms of resistance to fire, plant communities show high ecological plasticity, seed develop (serotinous cones), adult tolerance (thick barks) and vegetative regeneration (Tapias *et al.*, 2004).

Predicted climate change scenarios make Mediterranean ecosystems especially vulnerable, being considered a hotspot of global change impacts and risks. Temperature rise and rainfall decrease are expected to rise the drought risk and consequently forest wildfires, increasing soil erosion and reducing the natural regeneration of Mediterranenan forests (Lindner *et al.*, 2010).

Ecology of Mediterranean pines

Pine species are widely distributed across the Mediterranean basin, where are main components of the landscape and have a high economic and ecologic value (Gómez *et al.*, 2005). The high genetic variability and/or phenotypic plasticity exhibited by these pines explain their high colonizing abilities, and their fundamental role in vegetation dynamics in this region (Barbéro *et al.*, 1998; Chambel *et al.*, 2007). Among the Mediterranean pines found in the Iberian Peninsula, *Pinus pinaster* Ait. and *Pinus halepensis* Mill. are the most representative, covering respectively ca. 1505000 and 1182000 ha (Ruiz *et al.*, 2009).

(a) *Pinus pinaster* (b) *Pinus halepensis*

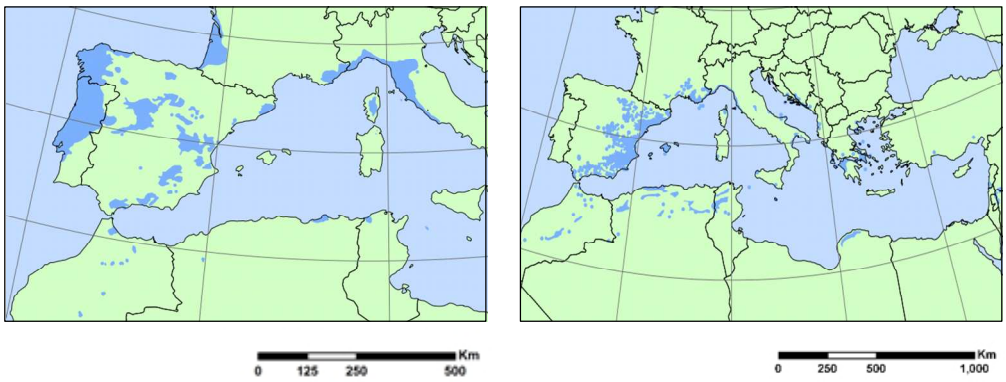


Figure 1 | Distribution area of (a) *Pinus pinaster* and (b) *Pinus halepensis*. EUFORGEN 2009, www.euforgen.org.

Maritime pine (*P. pinaster*) is naturally distributed in the Western Mediterranean, from southwestern Europe (France, Portugal, Spain, and Italy) to northwestern Africa (Algeria, Tunisia and Morocco) (Peñuelas and Ocaña, 2000) (Figure 1). This pine forms some of the most extensive and variable native forests in Spain, and part of this variation is likely tied to past fire regimes (Keeley *et al.*, 2011). It grows from the sea level to 2000 m, and tolerates a wide range of soils and climates, which has lead to its high genetic variation (Alía and Moro, 1996). Baradat & Marpeau (1988) defined three main genotype groups of this pine species: Atlantic, Mediterranean and Maghrebian, which have been later confirmed by molecular genetic tools (Bucci *et al.*, 2007; Rodríguez-Quilón *et al.*, 2016).

Aleppo pine (*P. halepensis*) occurs all around the Mediterranean basin, mainly along the coast and exceptionally in inland Spain, Tunisia and Italy (Gómez *et al.*, 2005). The biggest populations are found in Eastern Spain, Provence, Greece, Morocco and Algeria (Peñuelas and Ocaña, 2000) (Figure 1). This pine is the less exigent among the Mediterranean species and shows a great tolerance to elevated temperatures and drought, as well as to clay and gypsum soils. It appears at low altitudes to 800-900 m in the Iberian Peninsula, and develops well with 500 mm precipitation supporting levels up to 200-250 mm and even 5 months without rainfalls (Peñuelas and Ocaña, 2000).

From the phylogeographical point of view, *P. halepensis* appeared in the Iberian Peninsula as a result of a relatively recent colonization and therefore a loss of genetic diversity from the relictual Greek population has been observed, whereas *P. pinaster* presents high levels of genetic diversity and its presence in the Peninsula is much longer (Gómez *et al.*, 2005; Grivet *et al.*, 2009). Both tree species have a scattered distribution due to ecological disturbances (e.g.,

wildfires) and habitat fragmentation caused by long-term human impact in the Mediterranean basin.

GENERAL OBJECTIVES AND HYPOTHESES

The general objective of this thesis is to study the structural and functional responses of belowground microbial communities to principal biotic and abiotic factors in Mediterranean pine forests ecosystems, i.e., tree species identity and/or genotype, seasonality, edaphic compartment, fire recurrence. Possible mechanisms governing these plant-microbial-environment feedbacks have been explored by using enzymatic and spectral infrared (NIRs/MIRs) soil analyses, as well as metagenomic (454 GS-FLX pyrosequencing, Miseq) and phylogenetic approaches (Figure 2).

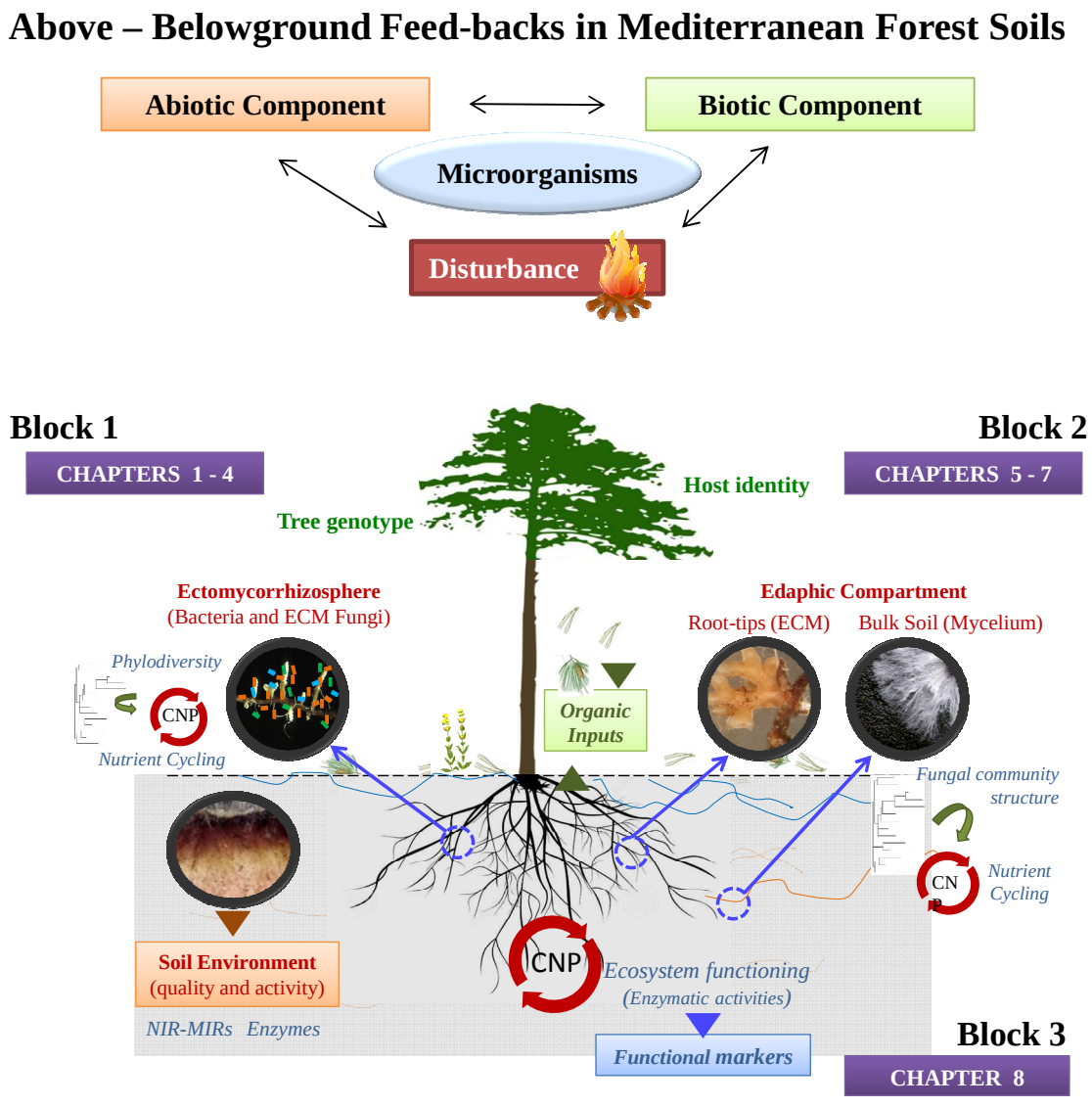


Figure 2 | Conceptual scheme of the thesis.

The general hypotheses of this work are:

1.- The overall quality of soils will be determined by the tree genotype or the pine species, as well as by context-dependent factors such as the site, the season and the fire regime.

2.- The tree host together with spatial-temporal factors will shape the diversity and assemblage of belowground fungal communities, with further functional consequences.

3.- The tree host identity and/or genotype, which reflect different phenotypes e.g., productivity and fire tolerance, will structure their associated microbial communities printing a phylogenetic signal that will favour specific microbial guilds.

4.- Tree genotype, seasonal or fire-induced effects on soil quality will affect the ecosystem functioning directly and through modulating the phylogenetic structure of belowground microbial communities.

In order to address these predictions, this thesis was structured in main Blocks of contents corresponding to three different experimental designs (Figure 2).

In the first Block, we tested the effect of biotic (tree genotype) and abiotic (site and season) factors on the soil properties, as well as on the structure and functioning of fungal and bacterial communities (Chapters 1-4) (Figure 2). We studied distinct *Pinus pinaster* genotypes in three common garden experiments established by the Spanish Forest Patrimony of State in Central Spain, in 1957. These replicated common gardens provided us a unique chance to test our hypotheses and to extract conclusions in a long-term scale.

In the second Block, we analysed the effect of the fire regime and the pine species identity on the soil properties, together with the structural and functional responses of the fungal communities settled in two edaphic compartments, i.e., root-tips and bulk soil (Chapters 5-7) (Figure 2). We analysed root and soil samples from natural populations of *P. pinaster* and *P. halepensis* located in eastern Spain and subjected to contrasted fire regimes.

Finally, in the third Block, we developed a new functional and taxonomic molecular marker from a single-copy gene indicative of the fungal secretome, which can be further used to analyse (i.e. by metatranscriptomics) the detailed functional response of fungal taxa to the environment (e.g., fire recurrence) (Chapter 8) (Figure 2). Indeed, this Block leaves a clear door opened for continuing this thesis work in the future.

The thesis is structured in eight chapters with the following specific objectives:

Chapter 1. Tree genotype and seasonal imprints on soil quality and functioning in Mediterranean pine forests

The aim of this chapter was to evaluate the impact of the tree genotype and the season on the global quality of soils and on relevant ecosystem processes such as C turnover and mobilization of nutrients. We evaluated the overall soil properties and soil functional responses beneath three *P. pinaster* genotypes differing in their productivities, at two seasons, by using NIR/MIR spectroscopy and enzymatic approaches.

Chapter 2. Functional outcomes of fungal community shifts driven by tree genotype and spatial temporal factors in Mediterranean pine forests

In this chapter, we aimed to determine the influence of biotic (i.e., tree genotype) and abiotic (i.e., season and site) factors on the α/β-diversity and species assemblage of soil fungal communities, and to explore whether structural shifts entailed functional consequences for the cycling of nutrients. We studied the fungal communities associated with three genotypes of *P. pinaster* by using high-throughput sequencing, and the soil environment was characterized by measuring physic-chemical soil properties and enzymatic activities.

Chapter 3. Plant genotype modulates nutrient cycling through its belowground microbial cloud

The objective of this chapter was to test if the phylogenetic community structure of symbiotic ectomycorrhizal fungi and rhizospheric bacteria was determined by the tree genotype regardless of the environmental (i.e., climatic and edaphic) conditions, and if these phylogenetic structural changes were further reflected in the ecosystem functioning related to nutrient cycles. To do that, we sequenced molecular markers and reconstructed the phylogeny of symbiotic ectomycorrhizal fungi and rhizospheric bacteria of different *P. pinaster* genotypic variants.

Chapter 4. Plant-soil feedbacks regulate nutrient cycling through the phylogenetic adjustment of ectomycorrhizal and saprotrophic fungal guilds

In this chapter, we aimed to analyse whether the soil quality generated under different *P. pinaster* genotypes and at different seasons determined the phylogenetic structure of soil fungal communities. We also targeted to define which phylogenetic groups within the ectomycorrhizal and saprotrophic fungal guilds were responsible for which specific functional processes. For this, we combined soil infrared spectral analyses together with phylogenetic methods and soil enzymatic approaches.

Chapter 5. The fire regime affects the quality and functioning of soils in Mediterranean pine forests

The objectives of this chapter were to compare the characteristics and enzymatic activity of forest soils of two Mediterranean pine species, and to determine if recurrent fires had left a signature in the overall soil quality and on processes affecting the cycling of nutrients in these forests. We studied soils from natural populations of *P. pinaster* and *P. halepensis* subjected to contrasted fire regimes, by using infrared spectral analyses and enzymatic approaches.

Chapter 6. Feedbacks of host tree and root-tip ectomycorrhizal communities in Mediterranean pine forests under distinct fire regime

In this chapter, we sought to examine whether the fire regime shaped the ectomycorrhizal fungal community associated with root tips of *P. pinaster* and *P. halepensis,* and if these fire-induced structural shifts affected functions linked with relevant ecosystem processes. We evaluated to which extent these responses were dependent on the pine species identity. We characterized the structure and functional traits of root-tip ectomycorrhizal fungal communities of *P. pinaster* and *P. halepensis,* in natural populations subjected to contrasted fire regime.

Chapter 7. Pine population genetics and fire regime shape the phylogenetic structure and functional traits of fungal communities in Mediterranean pine forests

The main objective of this chapter was to investigate if the pine population genetics, together with the fire regime, could print a phylogenetic signal on their associated fungal communities, and if these structural shifts had functional consequences. We comparatively studied root-tip and bulk soil compartments to evaluate the strength of the spatial distribution in fungal responses. We approached these objectives by studying the genetics of *P. pinaster* and *P. halepensis* populations subjected to contrasted fire regimes through single nucleotide polymorphism (SNP) analysis, combined with fungal phylogenetic analyses, soil infrared spectral measures, and enzymatic approaches.

Chapter 8. A new promising molecular marker to study the functional diversity of fungal communities: the GLYCOSIDE HYDROLASE 63 gene

In this final chapter, we sought to develop a new functional diagnostic molecular tool to monitor fungal communities in terms of structure, phylogeny and function, as a potential indicator of carbon cycling and secretome. To do that, we selected fungal genomes from the Mycocosm database to identify potential candidates genes, and developed primers to amplify the single-copy Glycoside Hydrolase Family GH63 gene, encoding α-glucosidases, from a large collection of fungal genomic DNAs. The efficiency of this primer pair was compared with other published markers and we compared the phylogenetic resolution of GH63 with one robust fungal phylogenetic marker.

Chapter 1

Tree genotype and seasonal imprints on soil quality and functioning in Mediterranean pine forests

INTRODUCTION

Forest soils are crucial for the global carbon cycle in Earth (Pan *et al.*, 2011). The physical, chemical and biological processes that take place in soils have a deep impact on the sustainability of ecosystems (Huang *et al.*, 2005). Among these processes, the enzymatic activity of soil has an essential role in the cycling of nutrient and the decomposition of complex organic matter compounds mostly derived from plant inputs, e.g. litter and root exudates (Baldrian, 2014). Soil enzymatic activity is influenced by different abiotic and biotic factors such as pH, climate, plant organic contribution, substrate availability, and/or microbial community composition (Sinsabaugh *et al.*, 2008; Baldrian *et al.*, 2010; Kivlin and Treseder, 2014; Courty *et al.*, 2016). In Mediterranean forest ecosystems, characterized by hot dry summers and scarce precipitation, plant primary productivity and hence the dynamics of litter and soil decomposition are highly influenced by the climatic conditions, especially by the availability of water (Gallardo and Merino, 1992; Scarascia-Mugnozza *et al.*, 2000; Sardans and Peñuelas, 2005).

The quality of soil is defined, in to a great extent, by the type and recalcitrance of organic substances, especially the content of lignin and nitrogen in litter, which may determine the soil enzymatic activity influencing the decomposition rates and the cycling of nutrients (Sinsabaugh *et al.*, 2002; Theuerl and Buscot, 2010; Talbot *et al.*, 2012). But the practical evaluation of the overall soil quality still remains a challenging issue (Gholizadeh *et al.*, 2013). Because infrared spectroscopic techniques, i.e., near (NIR) and mid (MIR) infrared, are sensitive to specific molecular vibrations of both organic and mineral soil components, and accurately measure several physical, chemical and biological properties at the same time, they are widely recognized as suitable tools for assessing and monitoring the quality of soil (Chodak *et al.*, 2002; Joffre *et al.*, 2001; Viscarra Rossel *et al.*, 2006; Knox *et al.*, 2015; Ludwig *et al.*, 2015). Although inherent differences of the near and mid spectral regions occur, e.g. in the NIR region overlapping combinations and overtone peaks may appear, and in the MIR region many bands correspond to simple compounds (Bellon-Maurel and McBratney, 2011), both approaches have been thoroughly used to report soil quality (Akroume *et al.*, 2016).

In forest ecosystems, trees are underpinning species, whose intraspecific genetic variation can impact the structure and functioning of the entire ecosystem (Whitham *et al.*, 2003; Schweitzer *et al.*, 2004; Wimp *et al.*, 2005). *Pinus pinaster* Ait. is a keystone species in the Mediterranean Basin that forms one of the most extensive forests in Spain (Peñuelas and Ocaña, 2000). This pine species thrives in a range of environmental conditions, from Mediterranean to Atlantic climate regimes, which explains its ecological and genetic versatility and the wide use of this pine species for intraspecific studies (Bucci *et al.*, 2007; Grivet *et al.*, 2011). Different *P. pinaster* genotypes corresponding to three main populations settled in the Atlantic coast,

southeastern Spain, and northern Africa are clearly distinguishable (Baradat and Marpeau, 1988; Bucci *et al.*, 2007; Rodríguez-Quilón *et al.*, 2016).

The tree species (Aponte *et al.*, 2010; Chavez-Vergara *et al.*, 2014) and even the tree genotype (Treseder and Vitousek, 2001; Madritch and Hunter, 2002) can determine the litter chemistry with a strong impact on carbon and nutrient dynamics. Likewise, the seasonality and tree phenology may influence the quality and quantity of organic inputs in soil (Courty *et al.*, 2007; Šnajdr *et al.*, 2011). Based on these observations, we hypothesized that, together with the seasonal influence, contrasted genotypes of *P. pinaster* would determine the overall quality of the soils beneath them. Variations in soil quality were further predicted to lead to changes in relevant ecosystem processes involved in carbon turnover and mobilization of nutrients. The establishment of common gardens with representative genotypes of *P. pinaster* by the Spanish Forest Patrimony of State in 1967 (Alía and Moro, 1996; González-Martínez *et al.*, 2004) provided us a unique opportunity to test these hypotheses in a long-term scale field trial. Infrared spectroscopy and enzymatic approaches were used to evaluate the quality and functioning of soils, and additionally, the resolution of two spectroscopic techniques was analysed by comparing their overlapping spectral region.

MATERIALS AND METHODS

Experimental design and sampling

The study was carried out in ~45 year-old *P. pinaster* common gardens located in Cabañeros (39º 22'N, 4º 24'W), Riofrío (39º 8'N, 4º 32'W), and Espinoso del Rey (39º 36'N, 4º 48'W) in central Spain (Alía *et al.*, 1997) (Figure S1a). Site characteristics are summarized in Table S1.The common gardens were originally settled in a completely randomized block design with four blocks per site and trees from different geographic provenances (Alía and Moro, 1996). The *P. pinaster* genotypes Atlantic (Galicia, Spain), Mediterranean (Valencia, Spain) and African (Jbel Tassali, Morocco) were selected for this study, and have been previously demonstrated to be genetically and phenotypically different (Alía and Moro, 1996; González-Martínez *et al.*, 2004). The three selected tree genotypes showed different productivity i.e. diameter at breast height, consistently across sites (Figure S1b).

Samplings were carried out in spring and autumn 2012. In each season, three trees per provenance and block were selected by site (3 sites x 4 blocks x 3 genotypes x 3 trees=108 trees) (Figure S2). Four soil subsamples (10 x 20 cm cores, at N, S, E and W) were taken 1 m far from the trunk of each tree and pooled into a unique sample. Once in the lab, soil samples were homogenized and sieved at 2 mm for further analyses.

Soil properties and Near-Mid infrared spectroscopic analysis

At each season, sieved soils were pooled by tree genotype and block (n = 35 per season; due to the opening of a firebreak, some trees lacked in Espinoso del Rey for a complete factorial design of 36 samples; see Figure S2). Soil were analysed for soil water content by drying 5g at 65 ºC for 48 h. The rest of soils were air-dried.

Initially, soils were measured for different variables: pH (1:5, w:v in H_2O), electrical conductivity (1:5, w:v in H_2O), organic matter (OM) and total C (Walkley and Black, 1934), total N (Kjeldahl method), and extractable P and K determined by inductively coupled plasma spectrometry (Optima 4300DV, Perkin-Elmer). In a subsequent step, spectroscopic near-mid infrared analyses were conducted as an integrative measure of the soil quality (Cécillon *et al.*, 2009). Prior to conduct spectroscopic infrared analyses, dry soil samples were grinded and re-dried at 30 °C for 24 h. Soil aliquots of 20-30 mg were taken and three replicates per sample were analyzed by near-infrared (9997 to 2198 cm^{-1}, NIR) and mid-infrared (5498 to 549 cm^{-1}, MIR) scanning using a HTS-XT Bruker spectrometer (Vertex 70, NIR-MIR-MCT, Bruker Corporation, Billerica, MA), the two sensors overlapping in the 5498-2198 cm^{-1} region. The spectra were recorded at 1.9 cm^{-1} intervals. The full range provided by each sensor was used, and the infrared scanner recorded the absorbance spectra for each soil sample.

Soil enzymatic analyses

Extracellular enzymes are considered good proxies of soil functioning given that they are the proximal drivers of decomposition and nutrient cycling in soils (Sinsabaugh *et al.*, 2008). Thus, soil functioning was evaluated by measuring the activity of eight hydrolytic and oxidative microbial exoenzymes on soil samples following the methodology adapted from Mathieu *et al.* (2013). Seven enzymatic tests were based on fluorogenic substrate release, methylumbelliferone (MU) e.g β-glucosidase (EC 3.2.1.3) and cellobiohydrolase (EC 3.2.1.91) that release glucose and cellobiose respectively from cellulose; xylosidase (EC 3.2.1.37) which hydrolyses xylose from xylan; β-glucuronidase (EC 3.2.1.31) related to the hydrolys of β-d-glucuronic acid residues from the non-reducing terminal of glycosaminoglycan; phosphatase acid (EC 3.1.3.2) involved in the breakdown of phosphoric ester bonds by releasing phosphate ions; chitinase (EC 3.2.1.14) that hydrolyses glycosidic bonds in chitin; and L-leucineaminopeptidase (3.4.11.1) which is able to remove the N-terminal l-leucine from peptidic substrates, this last based on methylcoumarine (AMC) fluorogenic substrate release. The Lacasse (1.10.3.2) activity, involved in the oxidation of substrates such as phenols or lignin, was determined by a photometric assay based on ABTS substrate (2,2'-Azino-bis 3-ethylbenzo-thiazolin-6-sulfonic acid) as described by Mathieu *et al.* (2013). Measurements were carried out in a Victor microplate reader (Perkin-Elmer Life Sciences,

Massachusetts, USA), with 355/460 nm excitation/emission wavelengths for the fluorogenic assays, and 415 nm for laccase.

Statistical analyses

Independent matrices with soil NIR-MIR spectral data sets consisted of 70 samples and 4044 (NIR) or 2566 (MIR) parameters, each corresponding to a wave frequency within the respective spectrum ranges (Figure S3). NIR and MIR spectral data were mathematically transformed by calculating the first derivate after standard normal transformation (Reeves *et al.*, 2002). Each spectral data set was analyzed by mean comparison tests ($p<0.05$) to identify the frequencies or the range of wave frequencies that significantly differed among tree genotypes and seasons. To reduce the dimensionality of NIR and MIR spectral matrices, principal components analyses (PCA) were run with the function *dudi.pca* included in the ade4 R package. Additionally, PCA analyses of two specific spectral regions were run (Figure S3): 1) the NIR and MIR overlapping region 5498-2198 cm^{-1} (herein named Region A) was selected to compare the resolution of both spectroscopic technics, and 2) the region 3036-2376 cm^{-1} (herein named Region B) related to O-alkyl C (deriving primarily from cellulose and hemicelluloses but also from proteins and side chains from lignin), aromatic C (related to lignin) and carbonyl C (from aliphatic esters, carbonyl groups, and amide carbonyls) was chosen as a proxy of soil organic matter (SOM) quality (Terhoeven-Urselmans *et al.*, 2006; Ludwig *et al.*, 2008).

Enzymatic data and soil variables were tested for normality (Shapiro test) and homoscedasticity (Levene test), and log or square root transformed when needed. To check the impact of the tree genotype and the season (fix factors) on soil properties and functioning, soil variables, enzymatic activities as well as the first three principal components (axes 1 to 3) of NIRs/MIRs PCA analyses were separately analyzed by General Linear Mixed Models (GLMM) ($p<0.05$) with the site as random factor.

Additionally, to test the relation among soil properties and functioning, soil NIR and MIR spectral data sets were analyzed by nonmetric multidimensional scaling (NMDS) with the function *metaMDS* in vegan R package (Oksanen *et al.*, 2015), and the enzymatic activities were fit into the NMDS space with the *envfit* function for significant correlations. All statistical analyses were performed using the software R 3.1.1 (R Core Team, 2014).

RESULTS

Soil properties

The tree genotype and the season significantly affected the soil properties, without interaction (Table S1). Lower EC, N and K contents were observed under the Atlantic genotype compared with each or both Mediterranean and African genotypes (Table S1). A significant increase of RH, EC, and K, coupled with a P and C:N decrease were observed in autumn compared with spring (Table S1).

Soil quality: near-mid infrared spectroscopy

The influence of the tree genotype and the season on soils was illustrated through the near and mid-infrared spectra, which provided an overall view of the soil quality (Figure 1; Figure S4). Regarding the tree genotype, significant differences were detected for 80 and 126 NIR bands (out of a total of 4045) in soils under the Atlantic trees respect to the Mediterranean and the African ones, while 45 bands were different between soils under the two last genotypes (Figure S4a). The MIR spectrum showed significant differences in 20 and 31 bands between Atlantic and Mediterranean or African genotypes respectively, out of a total of 2567, and both Mediterranean genotypes differed in 15 bands (Figure S4a). Regarding the season, 574 NIR bands and 1019 MIR bands significantly varied between spring and autumn (Figure S4b). These tree genotype and season imprints on soil quality were clearly separated by principal component analysis (Figure 1). The influence of factors was further confirmed when PCA axes were modeled (Table 1). The genotype effect was consistent in spring and autumn as indicated by the non-significant interaction between factors (Table 1). The three first principal components of all NIR and MIR data explained the ~50-60 % of the total variance in soil quality (Table 1), similar to that observed when the overlapping Region A was separately analyzed. This percentage of variance explained notably increased (71.2 % NIR / 86.8 % MIR) when the partial Region B, indicative of organic matter quality, was analyzed (Table 1).

As showed by the total NIR spectrum, the soil quality under the Atlantic genotype clearly differed from those beneath the Mediterranean and African trees (Axis-2), and the season affected also significantly the soil quality (Axis-3) (Figure 1a; Table 1). However, although the season effect was evidenced by MIR, the influence of tree genotype was not significant (Table 1; Figure 1a). When the overlapping Region A was analyzed, a significant tree genotype effect was observed in both spectra (Table 1): NIRs (Atlantic different from Mediterranean and African genotypes; Axis-2 in Figure 1b) and MIRs (Atlantic different from African trees; Axis-3 in Figure 1b). Concerning the Region B, both NIR and MIR approaches provided similar results revealing

significant differences between the Atlantic genotype with respect to the two other genotypes (Axis-1 for NIR and Axis-3 for MIR) (Table 1; Figure 1c).

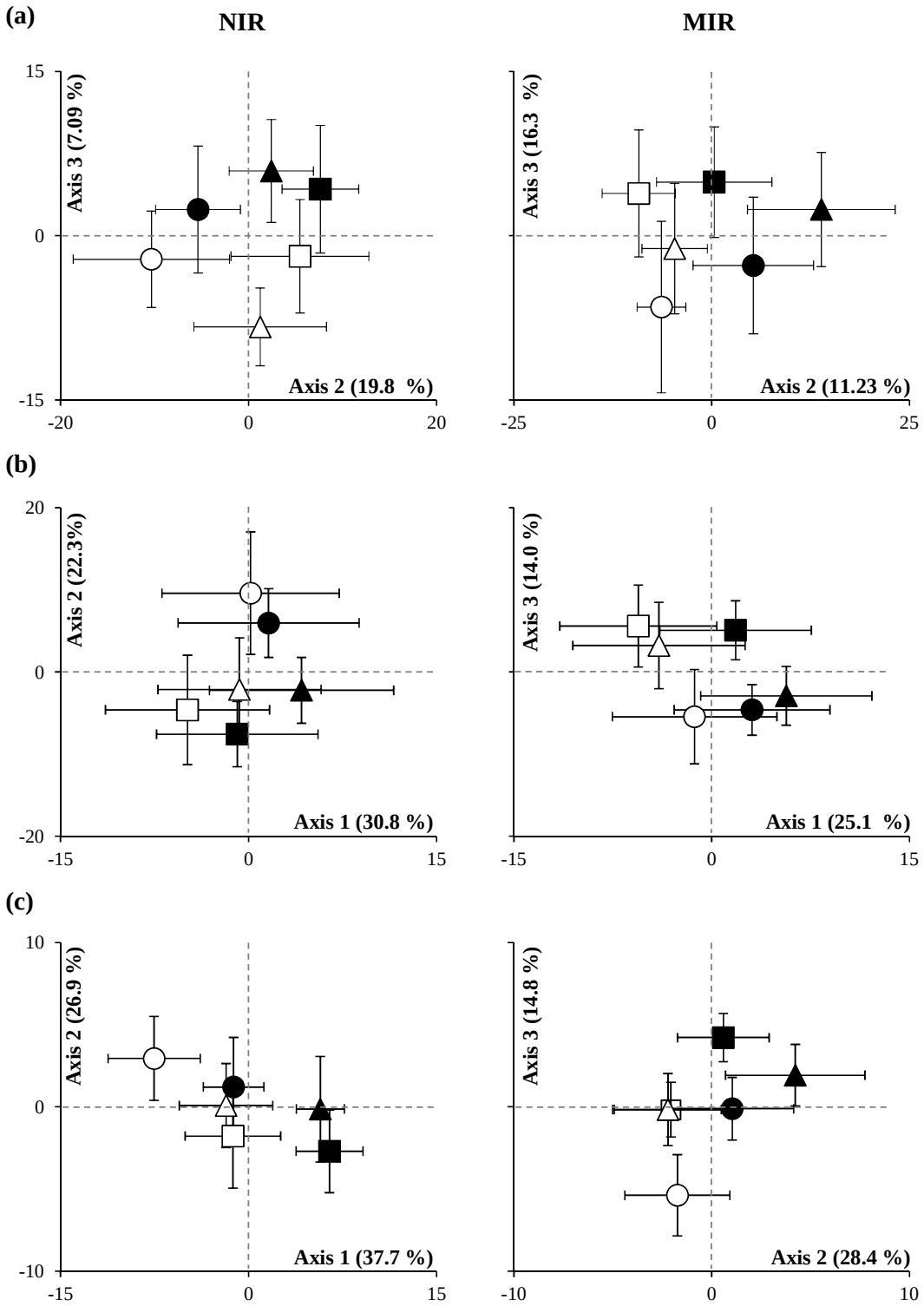


Figure 1 | Principal component analyses of near NIR (left) and mid MIR (right) infrared spectral data: (a) total data, (b) NIR/MIR overlapping region 5498-2198 cm^{-1}, and (c) NIR/MIR partial region 3036-2376 cm^{-1}, by tree genotype (circle = Atlantic; triangle = Mediterranean, square = African) and season (white = spring; black = autumn). Percentages in axes indicate the variance explained by each axis.

Table 1 | Effects of tree genotype (Gen) and season (Sea) on the near NIR and mid MIR soil infrared spectra analysed by General Linear Mixed Models, with site as random factor. Principal Correspondence Analyses (PCA) axes of total and partial regions of the spectra were considered as response variables, the percentage of the explained variance are shown in brackets (F values; *p>0.05; **p<0.01;***p<0.001).

	NIR				MIR		
	Gen	**Sea**	**G x S**		**Gen**	**Sea**	**G x S**
Total (9997-2198 cm^{-1})							
PCA Axis 1 (34.9 %)	2.49	1.12	0.23	*PCA Axis 1 (27.9 %)*	0.67	0.01	0.22
PCA Axis 2 (11.2 %)	**5.82****	0.71	0.12	*PCA Axis 2 (19.8 %)*	0.94	**6.88***	0.29
PCA Axis 3 (7.1 %)	0.20	**4.94***	0.65	*PCA Axis 3 (16.3 %)*	2.08	0.89	0.08
Region A (5498-2198 cm^{-1})							
PCA Axis 1(30.8 %)	0.86	**5.6***	0.54	*PCA Axis 1 (25.1 %)*	0.05	**10.86****	0.52
PCA Axis 2 (22.3 %)	**5.6****	0.45	0.11	*PCA Axis 2 (22.4 %)*	1.06	**6.12***	0.24
PCA Axis 3 (11.0 %)	0.20	0.09	0.96	*PCA Axis 3 (14.0 %)*	**3.95***	0.49	0.58
Region B (3036-2376 cm^{-1})							
PCA Axis 1 (37.7 %)	**5.14****	**13.67****	0.05	*PCA Axis 1 (43.6 %)*	1.02	2.95	0.99
PCA Axis 2 (26.9 %)	2.9	0.65	0.14	*PCA Axis 2 (28.4 %)*	0.22	**4.66***	0.46
PCA Axis 3 (6.6 %)	0.25	0.27	0.4	*PCA Axis 3 (14.8 %)*	**5.48****	**11.57****	0.7

Soil functioning: enzymatic activities

Almost all enzymatic activities were affected by the tree genotype and/or the season (Table S2). Regarding the enzymes degrading cellulose and labile sugars, the lowest cellobiohydrolase activity was found under the Atlantic tree genotype in spring (Figure 2a-b), while contrarily, a general higher activity of enzymes degrading hemicellulose (i.e. xylosidase and glucuronidase) was observed under this tree genotype (Figure 2c-d). In autumn, the laccase activity (involved in the oxidation of substrates such as polyphenols or lignin) and the phosphatase activity (involved in phosphorous mobilization) were significantly higher under the Mediterranean tree genotype compared with the African (Figure 2e-f). Leucine (involved in N mobilization) was significantly higher under the Atlantic tree genotype, particularly in spring (Figure 2g-h).

Concerning the factor season, the cellulose-degrading enzymes significantly increased in autumn only under the Atlantic tree genotype (Figure 2a-b). Hemicellulose degrading enzymes together with laccase always peaked in autumn (Figure 2c-e). About the N cycle, leucine was significantly higher in spring than autumn under the Atlantic and African trees (Figure 2g-h). Significant correlations between soil quality and soil functioning were revealed by the NMDS analyses, especially with the NIR spectrum (Figure 3).

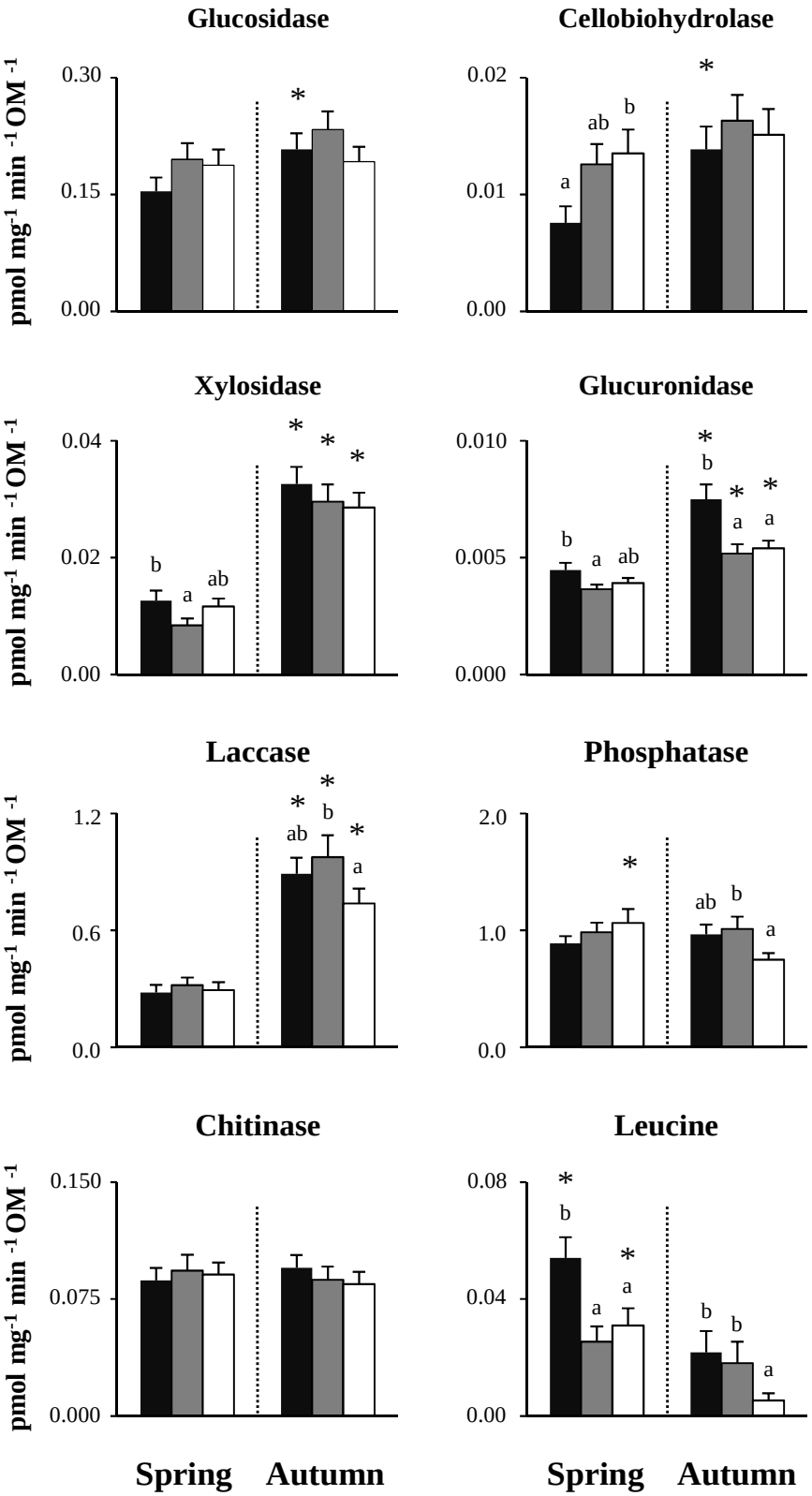


Figure 2 | Tree genotype and season effects on soil enzymatic activities. Values are means ± SE. Different letters denote significant differences among genotype treatments for a given season, while asterisks denote significant differences between seasons for a given tree genotype, according to LSD test ($p<0.05$). Bar colors indicate *Pinus pinaster* genotypes: black = Atlantic, grey = Mediterranean, white = African.

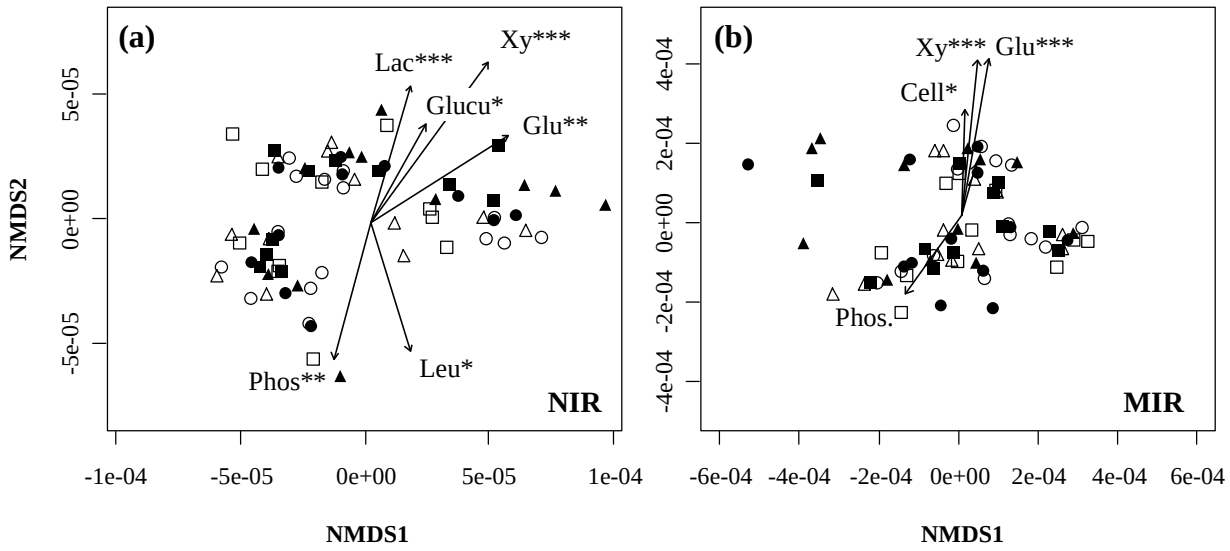


Figure 3 | Nonmetric multidimensional scaling (NMDS) of (a) NIR near (k=2; stress=0.09; R^2=0.99) and (b) MIR mid (k=2; stress=0.11; R^2=0.99) infrared spectral data by tree genotype (circle = Atlantic; triangle = Mediterranean, square = African) and season (white = spring; black = autumn). Vectors: strength and direction of enzymes (Glu = glucosidase; Cell = cellobiohydrolase; Xy = xylosidase; Glucu = glucuronidase; Lac = laccase; Phos = phosphatase; Chi = Chitinase; Leu = leucine) weight on the NMDS; .p<0.08; *p<0.05; **p <0.01; ***p<0.001.

DISCUSSION

The NIR-MIR spectroscopy provided an overall view of the soil quality and allowed to detect imprints of the studied biotic (tree genotype) and abiotic (season) factors. Because NIR and MIR spectra reflect a set of soil constituents that can be determined simultaneously (Terhoeven-Urselmans *et al.*, 2006; Viscarra Rossel *et al.*, 2006; Ludwig *et al.*, 2008, 2015), their use has been proposed as integrative soil quality variable suitable to discriminate between different levels of an ecological factor (Cécillon *et al.*, 2009; Akroume *et al.*, 2016). These authors suggested the use of the principal components from a multivariate analysis as a good proxy of the global status of soils without any previous calibration. Moreover, Reeves et al. (2000) concluded that infrared approaches might be appropriate where high precision in the components identification is not required. Due to the large quantity of information provided by these techniques, and for more accurate predictions of soil attributes, the partitioning of the spectra in our study was revealed to be highly informative, as previously pointed out (Knox et al., 2015). Compared with MIR, measures of the total NIR spectrum properly revealed the tree genotype effect on soils, although when the spectra were narrowed, both technics captured the effect of the tree genotype and the season with more variance explained. In general, the MIR region is assumed to be more suitable than the NIR one to carry out fine-scale analysis due to the higher incidence of spectral bands, as well as the higher intensity and specificity of the absorption features (Gholizadeh *et al.*, 2013). MIRs is assumed to be a suitable predictor for organic matter composition, while NIRs is commonly used to predict carbon and nitrogen soil stocks (Ludwig *et al.*, 2008; Soriano-Disla *et al.*, 2014). However, these assumptions are still controversial and the combined use of NIR and MIR for a more efficient soil characterization is recommended (Viscarra Rossel *et al.*, 2006; Akroume *et al.*, 2016).

As hypothesized, the tree genotype clearly impacted the overall soil quality, independently of the season effect, which also exerted a neat influence on soil. These effects were particularly visible in the overlapping region of the NIR-MIR spectra and in the representative organic matter region, i.e. recalcitrant organic compounds such as lignin or cellulose (Terhoeven-Urselmans *et al.*, 2006; Ludwig *et al.*, 2008). In general, the soil quality and the functioning beneath the Atlantic trees were different to that under the other genotypes, probably in relation to different litter properties. Indeed, the quantity, chemical composition and properties of litter, mainly leaf and root inputs, are among the main factors influencing the formation of SOM in terrestrial ecosystems (Scholes *et al.*, 1997; Wang *et al.*, 2010; Stewart *et al.*, 2011; Mueller *et al.*, 2015). Differences in litter qualities have been demonstrated at interspecific (Conn and Dighton, 2000; Fujii *et al.*, 2015; Wang *et al.*, 2016) and at intraspecific level (Madritch and Hunter, 2002; Madritch *et al.*, 2006; LeRoy *et al.*, 2012), and recognized as a consequence of genetic

expression (Allan *et al.*, 2012). Litter quality controls litter decay and soil functioning through its effect on the belowground microbial community (Madritch and Lindroth, 2011; Cotrufo *et al.*, 2013; Chavez-Vergara *et al.*, 2014). In fact, we observed that hemicellulose degradation and organic N mobilization were functions particularly active in soils beneath the Atlantic trees, while lignin degradation or P mobilization were particularly enhanced under the Mediterranean trees; on the other hand, less cellobiohydrolase activity was recovered in soils beneath the Atlantic pines specially in spring, probably indicating differences in the rhizodeposits provided by the different tree genotypes, i.e. readily degradable compounds such as monosaccharides, which may stimulate enzyme activities via priming (Allison and Vitousek, 2005; Hernández and Hobbie, 2010).

Mediterranean climate is characterized by a marked seasonality within and inter-year (Keeley *et al.*, 2011). Thus, as expectable in our study, the soil quality was clearly different in spring and autumn. These results could be largely explained by the higher activity of trees in spring, coupled with litter fall and water availability in autumn. In fact, we found evident temporal variations in extracellular enzymes related to cellulose and hemicellulose and other C recalcitrant compounds that picked in autumn, as already reported by Criquet et al. (2000) in Mediterranean oak forests. Similarly, the expression of leucine aminopeptidase, an enzyme involved in N mobilization from peptides, was higher in spring. Increased plant photosynthetic rates determine belowground C allocation (Högberg *et al.*, 2010; Kaiser *et al.*, 2010; Žifčáková *et al.*, 2016), and fall fresh litter inputs also affect the quality of SOM (Šnajdr *et al.*, 2011). Together with abiotic variables such as light, temperature and moisture (Baldrian, Šnajdr, *et al.*, 2013; Ekblad *et al.*, 2013), all these factors are major seasonal drivers of microbial activity affecting nutrient cycling and decomposition processes. In fact, the input and quality of litter are thought to be main regulators of C and N sequestration in soil (Wardle et al. 2012).

Additionally, as drawn by the ordination analysis, the overall soil quality was correlated with the soil functioning. Substrate quality and nutrient limitation (i.e. tree litter), coupled with temperature and soil moisture (i.e. season), are recognized amongst the main environmental drivers of the carbon use efficiency and the ecosystem functioning, directly and/or indirectly impacting soil microorganisms (Manzoni *et al.*, 2012). Since different microbial groups differentially decompose and assimilate carbon compounds depending on their quality (Waldrop and Firestone, 2004; Wang *et al.*, 2016), the functional effects observed in our study may reflect cascading effects on microbial communities, which deserves further analysis.

Our field design with different common gardens and blocks, reveals the tree genotype and the season as major drivers of soil quality and functioning, especially of processes related to carbon and nitrogen cycles. Since the tree genotype effect appears practically independent on the

season, near and mid-infrared spectra and enzymatic approaches arise as useful technics to provide an overall view of soil quality and to decipher separate imprints of biotic and abiotic factors on forest soils.

SUPPORTING INFORMATION

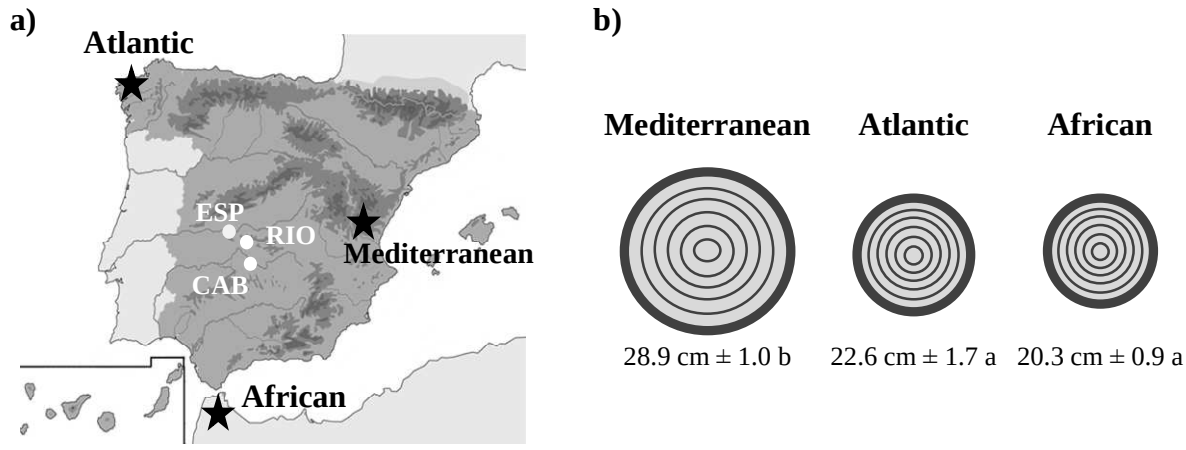


Figure S1 | (a) Genotypes of *Pinus pinaster* Ait. chosen for this study (asterisks) corresponding with Atlantic, Mediterranean, and African origin, and location of sampling sites (circles): CAB = Cabañeros, RIO = Riofrío, ESP = Espinoso del Rey. (b) Diameter at breast height (i.e. proxy of productivity) of the different tree genotypes at the time of the study ($F_{2,26}$ =13.9, P<0.001; tree genotype × site interaction: $F_{4,26}$ =1.65, P>0.1).

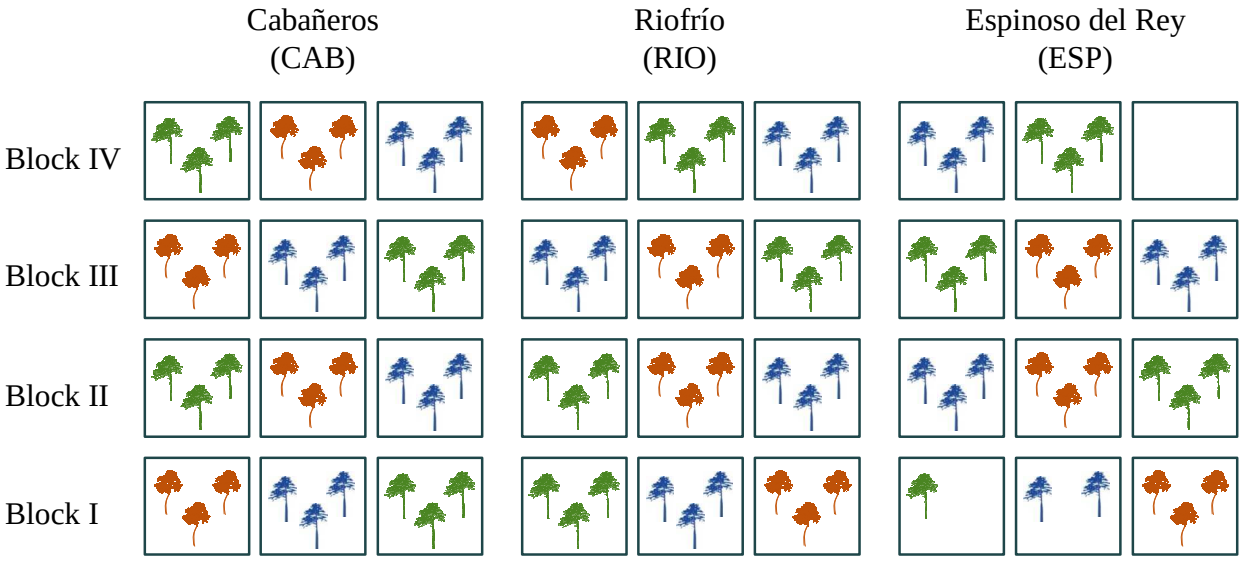


Figure S2 | Experimental common garden design of the present study, the three tree genotypes (green = Atlantic; blue = Mediterranean, orange = African) are replicated in four blocks by each site (Cabañeros, Riofrío and Espinoso del Rey). Due to the opening of a firebreak, some trees were lacking in Espinoso del Rey.

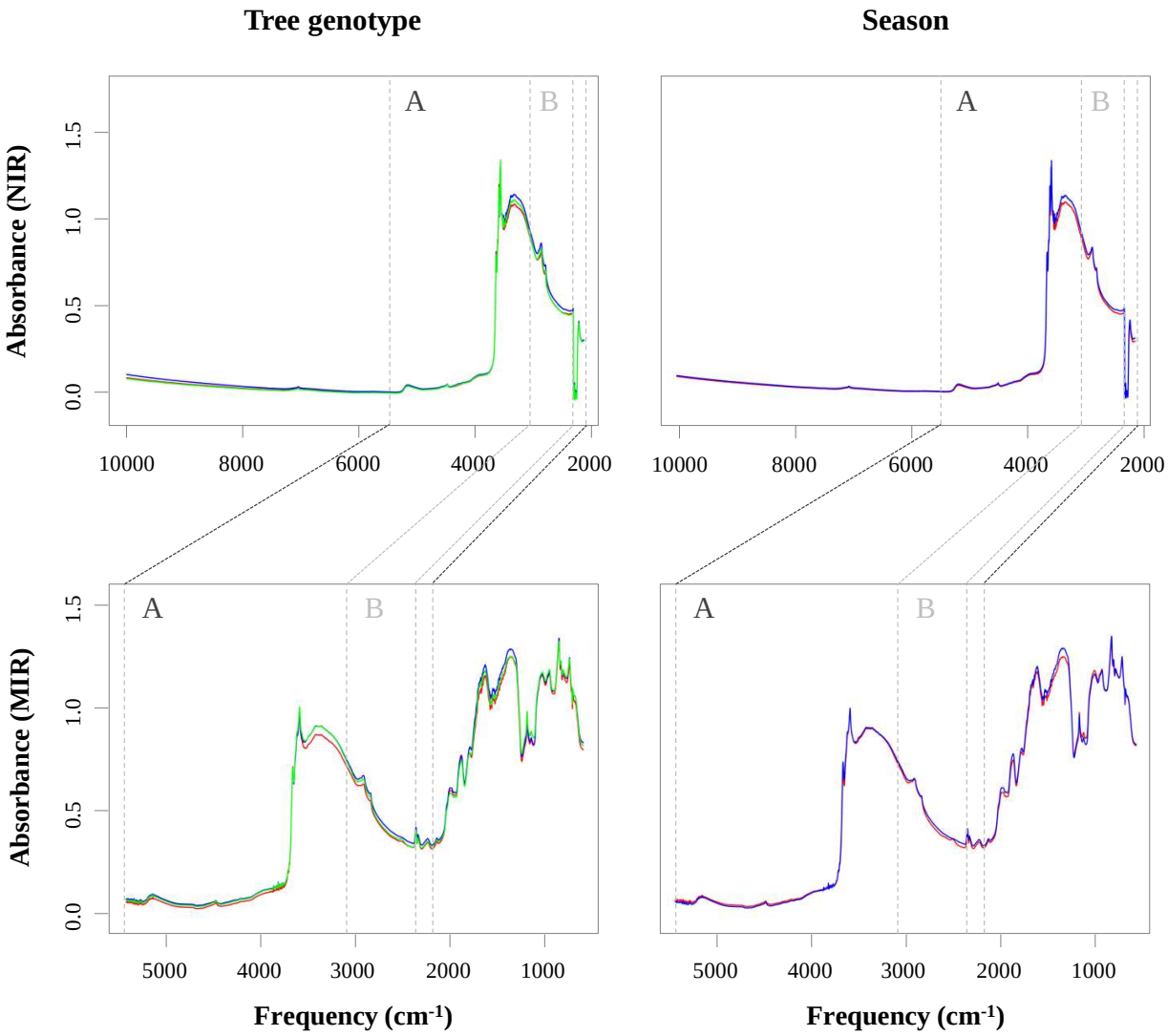
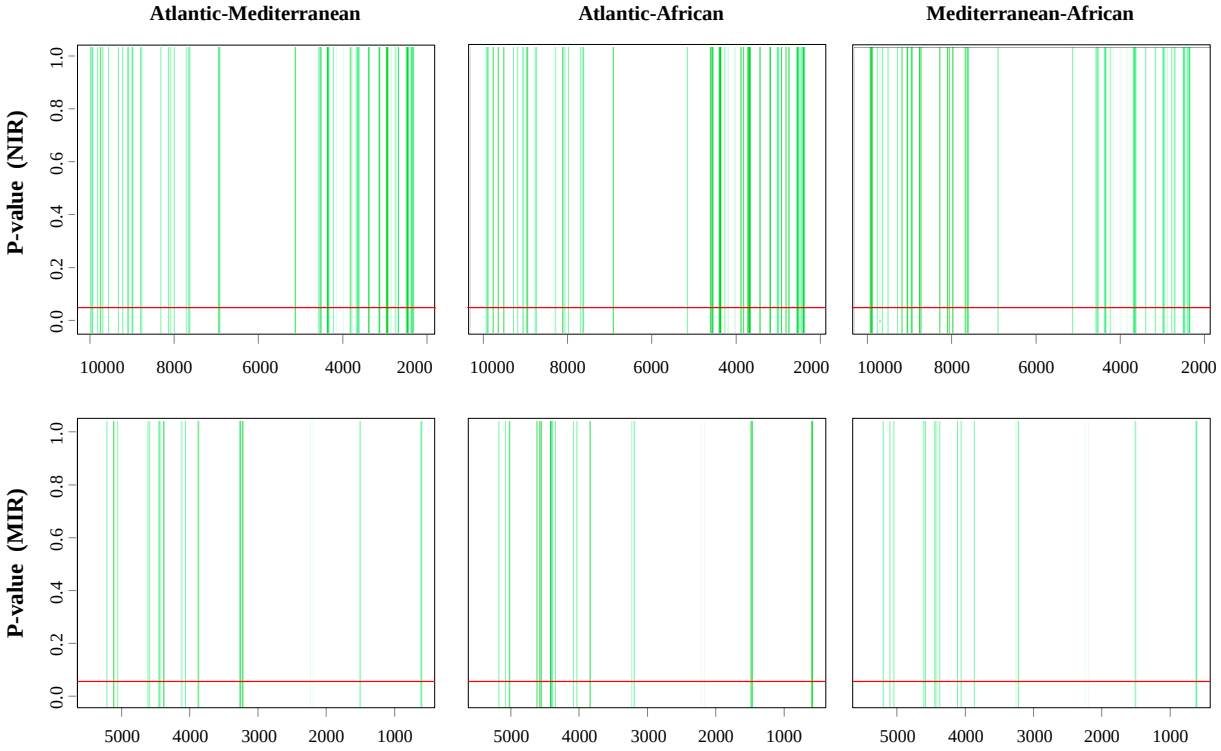


Figure S3 | Near and mid infrared spectral data by tree genotype (red = Atlantic; green = Mediterranean, blue = African), and season (blue = spring; red = autumn). The letter A showed the NIR-MIR overlapping region (5498-2198 cm^{-1}) and the letter B the region related to the organic matter quality (3036-2376 cm^{-1}).

(a) Tree genotype



(b) Season

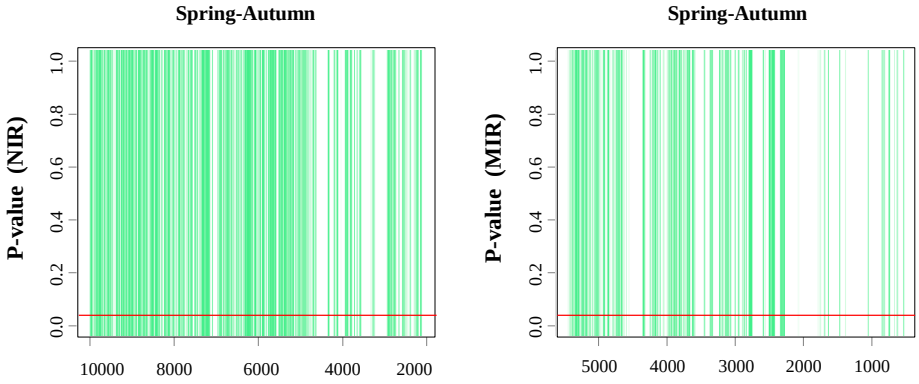


Figure S4 | Mean comparison test on near NIR and mid MIR infrared spectral data to identify the frequencies that significantly differ among (a) tree genotypes and (b) seasons. Significant frequency waves are indicated by green bars (p-value > 0.05).

Table S1 | Effect of genotype (Gen), season (Sea) and their interaction on soil properties analysed by General Linear Mixed Models with the site as random factor. F values and significance level: *p<0.05; **p<0.01;***p<0.001. [a]Pairwise comparisons among treatments for each factor by LSD test (p<0.05), Gen: Atlantic, Mediterranean, African; Sea: spring, autumn. Values = means ± SE. Within each factor, different letters indicate significant differences among treatments.

RH= relative humidity, EC = electric conductivity, OM = organic matter, N = nitrogen, P = phosphorous, K = potassium, C:N = carbon/nitrogen ratio.

Main test	RH (%)	pH	EC (µS/cm)	OM (%)	N (%)	P (mg/kg)	K (mg/kg)	C:N
Genotype	2.8	2.0	6.2**	2.6	3.9*	0.6	5.8**	0.24
Season	1163.6***	1.9	1163***	1.1	3.8	68.3***	35.7***	11.2**
Gen x Sea	1.0	0.2	1.5	0.27	0.3	0.14	0.22	0.40
[a]Pairwise test								
Atlantic	12.2 ± 1.6	5.1 ± 0.5	187.5 ± 26.0^{A}	7.3 ± 0.4	0.17± 0.0^{A}	4.1 ± 0.2	69.0 ± 8.2^{A}	26.0 ± 0.9
Mediterranean	13.5 ± 1.8	5.2 ± 0.5	231.0 ± 32.3^{B}	8.0 ± 0.4	0.18 ± 0.0^{AB}	3.9 ± 0.2	75.6 ± 8.9^{B}	25.3 ± 1.0
African	13.0 ± 1.8	5.1 ± 0.1	215.2 ± 30.3^{AB}	8.3 ± 0.4	0.19 ± 0.0^{B}	4.2 ± 0.3	77.7 ± 6.9^{B}	26.1 ± 1.1
Spring	4.9 ± 0.3^{A}	5.1 ± 0.0	73.7 ± 2.3^{A}	8.1 ± 0.3	0.17 ± 0.0	4.8 ± 0.1^{B}	65.2 ± 6.2^{A}	27.4 ± 0.4^{B}
Autumn	20.9 ± 0.4^{B}	5.2 ± 0.0	336.0 ± 9.9^{B}	7.6 ± 0.4	0.19 ± 0.0	3.3 ± 0.1^{A}	82.9 ± 6.4^{B}	24.1 ± 0.9^{A}

Table S2 | Effect of genotype (Gen), season (Sea) and their interaction on enzymatic activities analysed by General Linear Mixed Models with site as random factor. F values and significance level: .p<0.08; *p<0.05; **p<0.01;***p<0.001. [a]Pairwise comparisons among treatments for each factor by LSD test (p<0.05), Gen: Atlantic, Mediterranean, African; Sea: spring, autumn. Values = means ± SE. Within each factor, different letters indicate significant differences among treatments.

Main test	Glucosidase	Cellobiohydrolase	Xylosidase	Glucuronidase	Laccase	Phosphatase	Chitinase	Leucine
Genotype	1.38	2.51.	2.8 .	9.99***	2.29	0.61	0.073	1.09***
Season	4.36*	7.26*	164.78***	42.2***	120.53***	1.55	0.063	0.84*
Gen x Sea	0.82	1.24	0.84	2.67***	1.44	2.51	0.55	1.04***
[a]Pairwise test								
Atlantic	0.18 ± 0.01	0.011 ± 0.001 a	0.023 ± 0.002 b	0.006 ± 0.001 b	0.59 ± 0.06	0.93 ± 0.05	0.09 ± 0.006	0.038 ± 0.005 b
Mediterranean	0.21 ± 0.02	0.014 ± 0.001 b	0.019 ± 0.002 a	0.004 ± 0.000 a	0.65 ± 0.07	1.00 ± 0.07	0.09 ± 0.005	0.022 ± 0.004 a
African	0.19 ± 0.01	0.014 ± 0.001 b	0.020 ± 0.002 ab	0.005 ± 0.000 a	0.52 ± 0.05	0.91 ± 0.07	0.09 ± 0.02	0.018 ± 0.004 a
Spring	0.18 ± 0.01 a	0.011 ± 0.019 a	0.01 ± 0.001 a	0.004 ± 0.000 a	0.30 ± 0.02 a	0.98 ± 0.05	0.090 ± 0.005	0.037 ± 0.004 b
Autumn	0.21 ± 0.01 b	0.015 ± 0.001 b	0.03 ± 0.002 b	0.006 ± 0.000 b	0.87 ± 0.05 b	0.91 ± 0.05	0.089 ± 0.005	0.015 ± 0.004 a

Chapter 2

Functional outcomes of fungal community shifts driven by tree genotype and spatial temporal factors in Mediterranean pine forests

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INTRODUCTION

Fungal communities are key components of forest ecosystems that are involved in the biogeochemical cycling of nutrients and the productivity of trees. Saprotrophic fungi are primary decomposers, whereas ectomycorrhizal (ECM) fungi play main roles in decomposition and mobilization of nutrients (Lindahl *et al.*, 2007; Rineau *et al.*, 2013). Trees can invest up to a third of their primary production to maintain their associated ECM fungi (Smith and Read, 2008) in exchange for water and nutrients, and these fungal traits can be especially important under harsh environmental conditions. Fungi decompose the organic matter by the production of a wide set of extracellular enzymes capable of degrading complex cell wall biopolymers (Baldrian, 2014; Shah *et al.*, 2015). Fungal decomposition processes fluctuate seasonally in forest soils along with shifts in substrate availability and temperature and moisture variation (Baldrian, Šnajdr, *et al.*, 2013). Seasonal effects can be particularly pronounced in warm and water limited forests such as those in the Mediterranean area (Scarascia-Mugnozza *et al.*, 2000). Trees are main drivers of seasonality in resource availability for fungi via litter fall in autumn and belowground carbon exudation and uptake of nutrients in spring (Kaiser *et al.*, 2010; Voříšková *et al.*, 2014). Substrate supply in turn, stimulates the production of extracellular enzymes by fungi (Hernández and Hobbie, 2010; Navrátilová *et al.*, 2016), which can display distinct enzymatic traits depending on the environmental conditions and the fungal species (Courty *et al.*, 2005; Buée *et al.*, 2007; Bödeker *et al.*, 2009).

Microbial communities have been considered the extended phenotype of plant individuals, i.e. a heritable trait of a foundation tree species whose variation can impact the entire ecosystem (Whitham *et al.*, 2003; van der Heijden *et al.*, 2015). The plant and its microbiota are thus regarded as a unique holobiont system (Vandenkoornhuyse *et al.*, 2015; Hacquard, 2016). The characteristics of the dominant tree species in a site may delimit the fungal communities in soil through microclimatic variations and the organic inputs provided (Priha *et al.*, 1999; Kernaghan *et al.*, 2003), with potential effects on the ecosystem functioning. Within this context, for instance the poplar genotype determined the degree of colonization of different ectomycorrhizal fungal isolates (Tagu *et al.*, 2005), or the enzymatic activity of *Laccaria bicolor* ectomycorrhizas (Courty *et al.*, 2011). Other studies have revealed that the tree host genotype is a crucial factor structuring their associated fungi (Korkama *et al.*, 2006; Sthultz *et al.*, 2009; Courty *et al.*, 2011; Velmala *et al.*, 2013; Lamit *et al.*, 2016). Given the heterogeneous spatial-temporal distribution patterns of fungal communities, their dependence on the edaphic-climatic characteristics, the plant community composition and/or the tree host, assessing their interactive responses to biotic and abiotic factors is currently a major challenge in fungal ecology (van der Heijden *et al.*, 2015).

Pinus pinaster Ait. is a representative species in the Mediterranean Basin, covering approximately 1800000 ha in Spain (Villanueva, 2005). Three main geographic provenances, i.e. Atlantic, Mediterranean and African, with a clear genetic differentiation have been described (Baradat and Marpeau, 1988; Bucci *et al.*, 2007; Rodríguez-Quilón *et al.*, 2016). These different genotypes display a great phenotypic variability in traits such as cold, fire and drought tolerance, pest resistance, or growth and biomass production (Alía and Moro, 1996). We examined trees from the three main *P. pinaster* genotypes established in replicated long-term common garden plantations with the aim to (i) study the impact of biotic (i.e. tree genotype) and abiotic (i.e. season and site) factors on the diversity and assemblage of their associated fungal communities, and to (ii) explore whether structural shifts in fungal communities trigger functional responses affecting relevant ecosystem processes. Due to the heterotrophic nature of fungi, we predicted that under rather similar environmental conditions, tree genotypes differing in their productivity would support different taxonomic and functional fungal assemblages. Since carbon inputs are tightly linked to the phenology of trees (Buée *et al.*, 2005; Koide *et al.*, 2007) and the influence of roots (Cheng and Gershenson, 2007), fungal responses to the tree genotype would be dependent on the season, particularly affecting obligate biotrophic fungal guilds such as the ectomycorrhizal one. Expected structural shifts in fungal communities were further predicted to entail functional consequences related with the cycling of nutrients.

MATERIALS AND METHODS

Study sites and sampling

The study was conducted in common gardens established by the Spanish Forest Patrimony of State in 1967 with *P. pinaster* trees from different geographic origins (Alía and Moro, 1996). Three sites with rather similar environmental characteristics were located in central Spain: Cabañeros (39º 22'N, 4º 24'W), Riofrío (39º 8'N, 4º 32'W), and Espinoso del Rey (39º 36'N, 4º 48'W) (see Chapter 1). In all sites, the climate is Mediterranean, with cold wet winters and hot dry summers, mean annual temperature between 12-13.4 ºC and precipitation of 716-800 mm (Ninyerola *et al.*, 2005). The plant community is dominated by *P. pinaster*, with scattered *Quercus suber* L., *Quercus pyrenaica* Willd., and the understory composed of dispersed evergreen shrubs (e.g. *Erica arborea* L., *Cystus* sp., *Arcthostaphyllus uva-ursi* (L.) Spreng, *Lavandula stoechas* L., *Halimium umbellatum* (L.) Spach.).

Originally, all common garden plantations were settled in a completely randomized block design with four blocks and *P. pinaster* of different provenances (named "genotype" from herein), with 16 trees per each, separated of 2.5 m (Alía and Moro, 1996). Trees of contrasted geographic provenances, i.e. Atlantic (Galicia, Spain), Mediterranean (Valencia, Spain), and African (Jbel

Tassali, Morocco), were selected for this study. These tree genotypes have been previously demonstrated to diverge genotypically and phenotypically (Alía and Moro, 1996; Rodríguez-Quilón *et al.*, 2016). The three selected tree genotypes showed different productivity i.e. diameter at breast height, consistently across sites (see Chapter 1).

At each site, three trees per genotype and block were sampled in spring and autumn of 2012 (3 sites × 3 tree genotypes × 4 blocks × 3 trees × 2 seasons). Because a firewall created at one site (Espinoso del Rey) 6 trees lacked for a complete factorial design, and a total of 102 trees were sampled each season (see Chapter 1). Under each tree, litter was removed 1 m far from the trunk and subsamples were obtained by excavating 10 x 10 x 20 cm, at N, S, E and W orientations. The four subsamples per tree were joined in a single soil sample and kept at 4 ºC until processing. Once in the lab, soil samples were homogenized, sieved at 2 mm, and aliquots stored at -20 ºC for further molecular analyses. Remaining soil was air-dried for chemical analyses.

Soil analyses and enzymatic tests

Soil samples were pooled by tree genotype per site and experimental block into single composite replicates for chemical analyses (n = 35, per season). The relative humidity (RH) of soils was determined by drying at 65 ºC for 48 h. Other soil variables were measured, such as pH (1:5, w:v in H_2O), electrical conductivity (1:5, w:v in H_2O), organic matter (OM) and total carbon (C), total nitrogen (N) (Kjeldahl method), and extractable phosphorus (P) and potassium (K) determined by inductively coupled plasma spectrometry (Optima 4300DV, Perkin-Elmer).

Fungal community functioning was evaluated by measuring soil activities of eight hydrolytic and oxidative exoenzymes secreted by fungi, following the methodology adapted from Mathieu *et al.* (2013). Seven enzymatic tests targeting different nutrient cycling processes were performed e.g. β-glucosidase (EC 3.2.1.3), cellobiohydrolase (EC 3.2.1.91), implicated in cellulose degradation; xylosidase (EC 3.2.1.37), and β-glucuronidase (EC 3.2.1.31), involved in hemi-cellulose degradation; laccase (1.10.3.2) involved in the oxidation of recalcitrant substrates such as phenols or lignin; phosphatase acid (EC 3.1.3.2) mobilizing phosphorous; and chitinase (EC 3.2.1.14) and L-leucineaminopeptidase (3.4.11.1) involved in the mobilization of nitrogen. The Lacasse activity was determined by a photometric assay based on ABTS substrate (2,2'-Azino-bis (3-ethylbenzo-thiazolin-6-sulfonic acid) as described by Mathieu *et al.* (2013). The rest of tests were based on fluorogenic substrate release, i.e. methylumbelliferone (MU) or methylcoumarine (AMC) (for L-leucineaminopeptidase). Measurements were carried out in a Victor microplate reader (Perkin-Elmer Life Sciences, Massachusetts, USA), with 355/460 nm excitation/emission wavelengths for the fluorogenic assays and 415 nm for laccase. At each

season, enzymatic analyses were performed on single soil samples (n = 102), and data were thereafter pooled into composite replicates (n =35), as previously explained. All enzymatic activities were expressed in pmol min^{-1}mg of $soil^{-1}$.

DNA extraction, PCR and 454-pyrosequencing

Genomic DNA was extracted from 0.5 g of soil with the PowerSoil kit (MoBio, Carlsbad, CA, USA). The internal transcribed spacer region ITS-1 of the nuclear ribosomal DNA was amplified with the primer pair ITS1F-ITS2 (Gardes and Bruns, 1993) adapted for pyrosequencing as described by Buée *et al.* (2009). PCR amplifications (3 min 94 ºC, 30 cycles of 1 min 94 ºC, 30 s 53 ºC and 45 s 72 ºC, with a final step of 10 min 72 ºC) were conducted in a Verity Thermal Cycler (Life Technologies), and each sample amplified in three independent 20 µl reactions, each containing 2 µl of 10x polymerase buffer, 2.4 µl of 25 mM $MgCl_2$, 1.12 µl of 10 mg ml^{-1} BSA, 0.4 µl of 10 mM nucleotide Mix, 0.4 µl of 10 mM forward/reverse primers (adaptor A-tag-ITS1F/adaptor B-ITS2), and 0.2 µl of AmpliTaqGold polymerase (5 U ml^{-1}) (Applied Biosystems, Carlsbad, CA, USA). Negative controls without DNA were included in all runs to detect possible contaminations. Independent reactions were combined per sample, and each PCR product was purified (UltraClean PCR clean-up kit of MoBio, Carlsbad, CA, USA), quantified (PicoGreen, Life Technologies, Carlsbad, CA, USA) and pooled in equimolar libraries (one per season) containing 35 uniquely tagged replicates, each resulting of pooling three samples by each tree genotype per site and experimental block. Pyrosequencing was carried out in a GsFLX-454 system (Roche Applied Biosystems, USA) in an external service (Parque Científico de Madrid, Spain).

Bioinformatic analyses

Sequences were de-multiplexed according to their tags, filtered and trimmed using the *fastq_filter* command and *fastq_truncqual* option of USEARCH v7.0.1001 (Edgar, 2013) and quality scores less or equal than 10 were eliminated. The ITS1 was extracted with the Fungal ITSx v1.0.3 (Bengtsson-Palme *et al.*, 2013) and partial ITS sequences shorter than 100 bp were discarded. De-replication of extracted ITS sequences was performed with the *derep_fulllength* USEARCH command. De-replicated sequences were then sorted by decreasing abundance, and singletons discarded with the *sortbysize* USEARCH command. The 92.3 % (166927) of the initial set of sequences (180921) was retained. Molecular operational taxonomic units (MOTUs) were generated from abundance-sorted sequences using the *cluster_otus* USEARCH command with a 97 % similarity threshold. Extracted ITS sequences, including singletons, were then mapped against the MOTU representative sequences using the *usearch_global* USEARCH command. Taxonomic assignation of representative sequences for each MOTU was done by using the Basic

Local Alignment Search Tool (BLAST) algorithm v 2.2.23 (Altschul *et al.*, 1990) against the UNITE database release 7.1 (Kõljalg *et al.*, 2013). Once taxonomic identification was achieved, fungal MOTUs were classed by their life style i.e. ectomycorrhizal, saprotrophic, endomycorrhizal, parasite, pathogen, lichen or unknown according to Tedersoo *et al.* (2014). The 454 .sff files and raw data were deposited in the Sequence Read Archive (SRA-NCBI, http://www.ncbi.nlm nih.gov/sra) as PRJNA324224.

Statistical analyses

All variables were verified for normality and homoscedasticity, and relations among them tested by Spearman correlation analysis ($p<0.05$). Alpha-diversity (i.e. number of MOTUs) of total and fungal guilds (i.e. life style, fungal phyla, families) was modelled by Generalized Linear Mixed Models (GLMM) with the tree genotype and season as fix factors, and the site as random factor, considering the number of reads as covariate. Relationships between fungal alpha-diversity (total and by fungal guilds i.e. different phyla and life styles) with soil properties and enzymatic variables were also tested by GLMM (Pinheiro *et al.*, 2014).

To identify fungal MOTUs significantly more represented across the different treatments, the Indicator Species Analysis (with MOTUs >100 reads) was carried out ($p<0.05$) (Cáceres *et al.*, 2013).

Bray-Curtis distance matrices of fungal species were calculated based on the abundance matrix of MOTUs, previously normalized (i.e. DESeq variance stabilization; McMurdie and Holmes 2014) (Anders and Huber, 2012). Over this matrix, fungal Beta-diversity was calculated (Anderson *et al.*, 2006; Oksanen *et al.*, 2015), considering the factors genotype, season, site and their interactions. Fungal community assemblage was analyzed by multivariate analysis of variance (PERMANOVAs) and nonmetric multidimensional scaling (NMDS) analysis (Oksanen *et al.*, 2015). All statistical analyses were carried out with the R software v3.0.2 (R Core Team, 2014).

Structural Equation Models

To get an integrative outline of the relationships among fungal diversity, function and edaphic properties, structural equation modelling (SEMs) was performed. An aprioristic model explicitly including the causal relationships among variables was built based on literature (Flores-Rentería *et al.*, 2016) (Figure S1). Our sample size was relatively small (n = 70) and the predictors included in the model were restricted, as recommended (Shipley, 2002). Enzymatic activities, representative of different nutrient cycles (i.e. glucosidase, cellobiohydrolase, xylosidase, glucuronidase, laccase for C; leucine and chitinase for N; acid phosphatase for P), were analyzed in separated models, and the Shannon index, which integrates frequency and abundance, was

chosen as fungal diversity variable. It was hypothesized that fungal diversity, as well as the tree productivity (represented by the diameter at breast height, DBH), and edaphic conditions (e.g. RH, pH, C/N, OM and P) would determine the ecosystem functioning (Figure S1). Causal relations and correlations among biotic and abiotic variables were included in the model, and all direct and indirect relations between exogenous and endogenous variables tested. Several models including all explicative variables were run, and the best fitted chosen according to the setting between the covariance in observed and expected data (i.e. goodness-of-fit $\chi 2$). Standardized path coefficients were estimated by using the maximum likelihood algorithm (Shipley, 2002). Model fit to data was evaluated by root mean square error of approximation (RMSEA) and the goodness-of-fit index (GFI) and the Bentler and Bonett's normed-fit index (NFI). SEMs were built with AMOS v.20.0 software (IBM Corporation Software Group, Somers, NY).

RESULTS

Sequencing yields and identification of fungi

A total of 1412 MOTUs were obtained (Figure 1a; Table S1). Almost half of MOTUs were shared by the three tree genotypes, while close to 9 % were common to each two genotypes, or exclusively found under one tree genotype (Figure 1a). The 65.7 % of MOTUs were present at both seasons, and the 15.5 % and the 18.8 % found in spring and autumn, respectively. Almost a third of MOTUs was found in all sites (Figure 1a; Table S1).

Sequencing and MOTUs yields per sample were quite homogeneous across treatments (Table S1). The 81.7 % of MOTUs, representing approximately the 99 % of reads, were assigned to phylum, e.g. 38 % Basidiomycota, 37.7 % Ascomycota, and 5.5 % Zygomycota. The 60.7 % of MOTUs was ascribed to family, the 50.4 % to genus, and the 27.5 % identified down to the species level. The life style of near the 60 % of MOTUs, representing the 93 % of reads, was inferred, most of which were saprotrophic (SAP, 47.4 %) and ectomycorrhizal (ECM, 44.6 %).

Among the 20 most abundant fungi, the ECM predominated together with two saprotrophic *Mortierella* sp. (Table S2). Certain MOTUs were preferentially associated with a tree genotype, while others were indicators of each season (Figure 1b; Table S3). Among the tree genotypes, the Atlantic showed the most divergent indicator species profile, while the Mediterranean and African were relatively similar (Figure 1b). Additionally, the tree genotype preferentially associated with certain fungi depending on the season and the site (i.e. genotype × season, genotype × site) (Table S3).

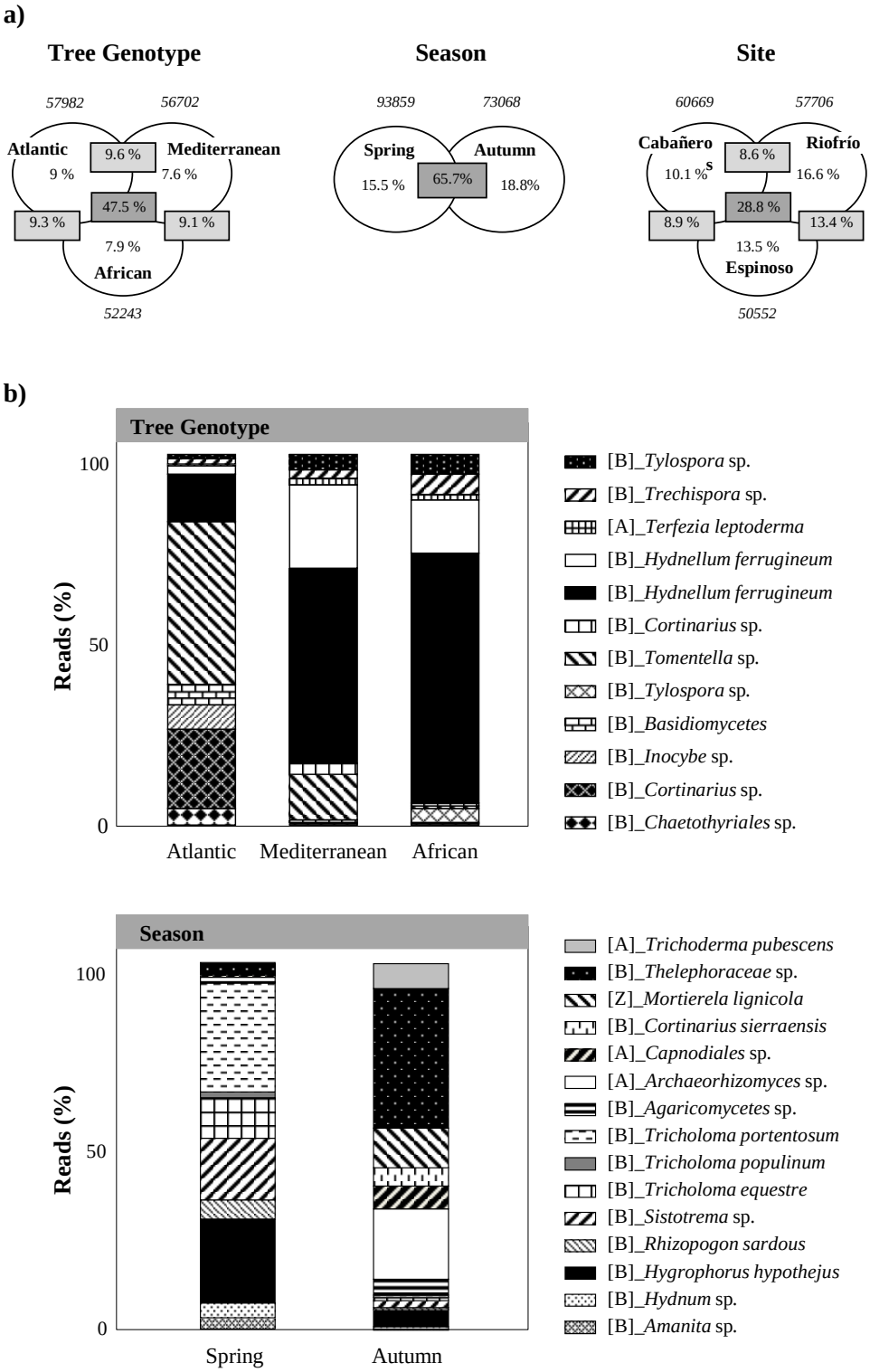

Figure 1 | (a) Number of sequences (cursive) and percentages of fungal Molecular Operational Taxonomic Units (MOTUs) by tree genotype (Atlantic, Mediterranean, African), season (spring, autumn), and site (Cabañeros, Riofrío, Espinoso del Rey). Inside squares are MOTUs shared by all (dark grey) or between each two treatments, while MOTUs exclusively found in a treatment are inside circles. (b) Indicator fungal species of different *Pinus pinaster* Ait. genotypes and seasons ($p<0.05$). [A] = Ascomycota; [B] = Basidiomycota; [Z] = Zygomycota; See Table S2 for additional information of indicator fungal species.

Fungal community structure

The tree genotype significantly affected the α-diversity of basidiomycetes (i.e. less α-diverse under the Atlantic trees), but not that of the overall community, or the rest of fungal guilds (Figure 2a). The season clearly affected the total fungal α-diversity (i.e. higher in autumn than spring) (Figure 2b); ascomycetes and zygomycetes kept this pattern, whereas basidiomycetes were equally α-diverse in both seasons (Figure 2b). By life style, the ECM fungi were more α-diverse in spring than autumn, whereas the saprotrophic fungi displayed the opposite pattern (Figure 2b).

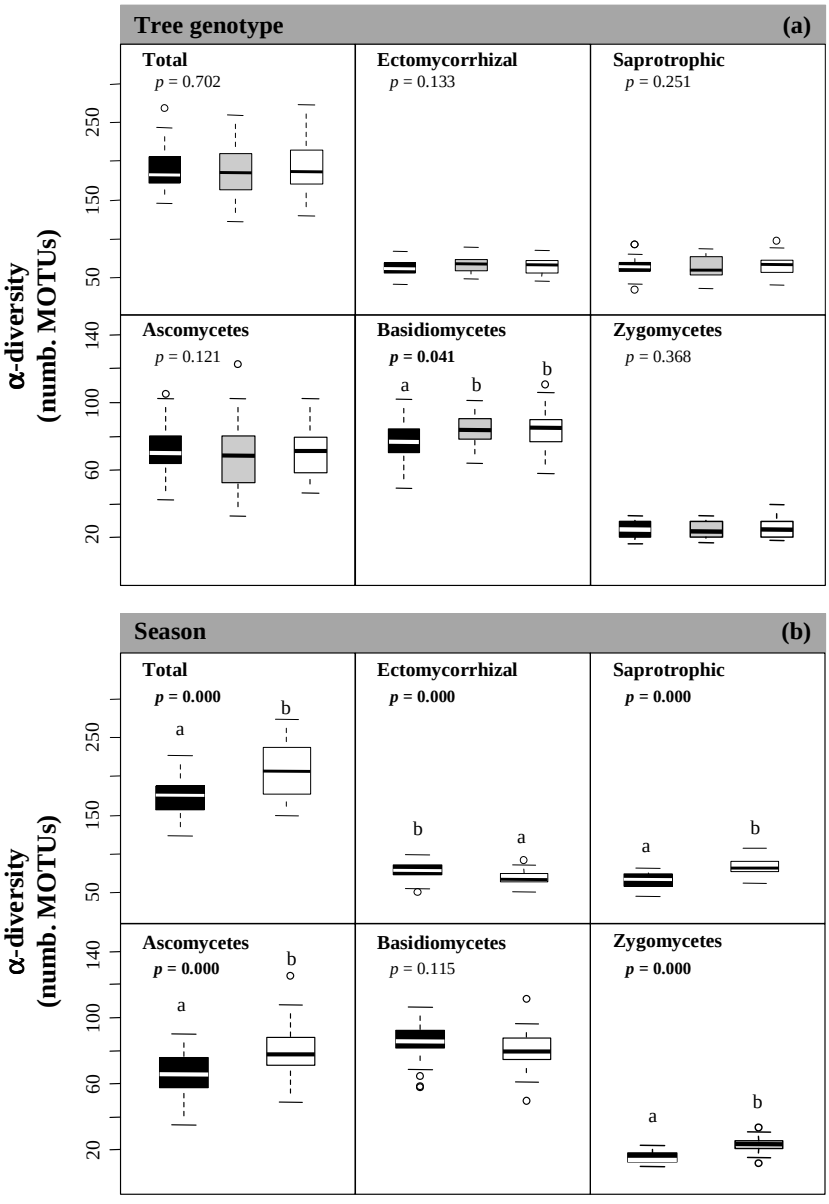

Figure 2 | Alpha-diversity of total fungal community and of representative fungal subgroups associated with (a) different *Pinus pinaster* Ait. genotypes (black = Atlantic; grey = Mediterranean, and white = African), and (b) at different seasons (black = spring, and white = autumn). Boxes represent the interquartile range (IQR) between first and third quartiles and the horizontal line inside is the median. Whiskers denote the lowest and highest values within 1.5 x IQR from the first and third quartiles, respectively. Within each graph, different letters denote significant differences among treatments according to the LSD test ($p<0.05$).

Focusing on lower taxonomic levels and consistently across sites, the tree genotype selectively affected the α-diversity of certain representative ectomycorrhizal fungal families, while others did not respond (e.g. Russulaceae, Thelephoraceae) (Table 1). For example, under the Atlantic trees, Atheliaceae and Entolomataceae were less α-diverse compared with the other tree genotypes, and with the Mediterranean one in the case of Bankeraceae, Sebacinaceae and Tuberaceae (Table 1); contrarily, Amanitaceae, Inocybaceae and Pyronemataceae were more α-diverse under the Atlantic than the African trees (Table 1). As previously pointed out by overall diversity results, the season had a significant effect that was specifically revealed on certain ectomycorrhizal families, generally more α-diverse in spring (Table 1), and on numerous saprotrophic families that peaked up in autumn (Table 1).

Table 1 | Alpha-diversity of representative fungal families and effects of tree genotype (G) and season (S) and its interaction (G x S) analysed by general linear mixed models with site as random factor. Main test results are shown in the first three columns (F values; *p<0.05; **p<0.01;***p<0.001), followed by post-hoc LSD test analysis (p<0.05) for tree genotype (Atl = Atlantic; Med = Mediterranean; Afr = African) and season; values = means +/- SE; for each factor, different letters denote significant differences (in bold). § = ectomycorrhizal families; ¥ = mainly represented by ECM species.

	Tree genotype	Season	G x S	Tree genotype			Season	
				Atl	Med	Afr	Spring	Autumn
Amanitaceae §	**5.2****	**17.3*****	**3.1***	**0.8 ± 0.1 b**	**0.6 ± 0.1 ab**	**0.5 ± 0.2 a**	**0.9 ± 0.1 b**	**0.4 ± 0.1 a**
Atheliaceae¥	**3.8***	2.5	0.0	**12.5 ± 0.8 a**	**14.5 ± 0.9 b**	**15.0 ± 1.0 b**	14.6 ± 0.7	13.3 ± 0.8
Archaeorhizomycetaceae	1.1	**18.5*****	0.6	3.0 ± 0.3	2.8 ± 0.4	2.9 ± 0.3	**2.3 ± 0.2 a**	**3.5 ± 0.2 b**
Bankeraceae §	**5.1****	0.0	0.0	**0.7 ± 0.4 a**	**1.8 ± 0.5 b**	**1.7 ± 0.4 ab**	1.5 ± 0.4	1.3 ± 0.3 a
Clavulinaceae §	0.3	0.2	0.2	1.0 ± 0.2	1.4 ± 0.3	0.9 ± 0.2	1.1 ± 0.2	1.1 ± 0.2
Cortinariaceae §	1.3	**4.5***	0.3	2.7 ± 0.3	2.6 ± 0.3	3.2 ± 0.4	**3.2 ± 0.3 b**	**2.5 ± 0.3 a**
Entolomataceae¥	**13.3*****	3.1	0.3	**0.4 ± 0.1 a**	**1.1 ± 0.1 b**	**0.8 ± 0.1 b**	0.8 ± 0.1	0.7 ± 0.1
Herpotrichiellaceae	2.7	**47.1*****	0.7	14.6 ± 0.9	13.7 ± 1.0	14.9 ± 0.8	**12.3 ± 0.6 a**	**16.5 ± 0.7 b**
Hygrophoraceae	0.5	0.3	0.5	1.0 ± 0.2	0.9 ± 0.2	0.9 ± 0.2	0.9 ± 0.1	1.0 ± 0.2
Hypocreaceae	1.3	**43.8**	0.9	1.6 ± 0.3	1.8 ± 0.3	1.9 ± 0.3	**0.9 ± 0.2 a**	**2.6 ± 0.3 b**
Inocybeaceae §	**3.4***	2.4	0.9	**9.3 ± 0.7 b**	**8.7 ± 0.6 ab**	**8.1 ± 0.70 a**	9.3 ± 0.6	8.1 ± 0.5
Mortierellaceae	1.3	**84.9*****	0.8	10.5 ± 0.8	10.6 ± 0.7	11.7 ± 1.0	**8.1 ± 0.4 a**	**13.8 ± 0.5 b**
Pezizaceae	1.7	2.3	2.2	2.4 ± 0.3	2.1 ± 0.4	1.9 ± 0.3	2.2 ± 0.3	2.1 ± 0.3
Pyronemataceae	**10.8*****	0.3	0.3	**1.7 ± 0.2 b**	**1.3 ± 0.2 ab**	**0.9 ± 0.2 a**	1.4 ± 0.2	1.2 ± 0.2
Rhizopogonaceae §	2.6	**8.7****	0.2	2.8 ± 0.2	1.9 ± 0.2	2.3 ± 0.2	**2.7 ± 0.2 b**	**2.0 ± 0.2 a**
Russulaceae §	0.9	3.0	0.8	5.9 ± 0.5	6.1 ± 0.6	6.8 ± 0.7	7.0 ± 0.6	5.5 ± 0.4
Sebacinaceae §	**7.9*****	0.1	1.0	**2.8 ± 0.5 a**	**4.8 ± 0.6 b**	**3.3 ± 0.4 ab**	3.8 ± 0.4	3.5 ± 0.4
Telephoraceae §	0.9	0.7	0.4	11.5 ± 0.7	12.1 ± 0.6	12.8 ± 0.9	12.4 ± 0.6	11.9 ± 0.6
Trichocomataceae	0.6	**7.9****	0.5	5.5 ± 0.4	5.3 ± 0.4	5.8 ± 0.4	**5.0 ± 0.3 a**	**6.0 ± 0.3 b**
Tricholomataceae	0.4	**6.9***	1.4	2.1 ± 0.3	1.8 ± 0.3	2.0 ± 0.3	**2.5 ± 0.3 b**	**1.4 ± 0.2 a**
Tuberaceae §	**6.9****	1.3	0.3	**1.1 ± 0.1 a**	**1.5 ± 0.1 b**	**1.4 ± 0.1 ab**	1.4 ± 0.1	1.3 ± 0.1
Umbelopsidaceae	2.2	**28.8*****	0.1	7.6 ± 0.4	6.7 ± 0.5	7.3 ± 0.5	**6.2 ± 0.3 a**	**8.2 ± 0.3 b**

All factors, i.e. tree genotype, season and site, significantly structured local soil fungal assemblages, with particularly strong spatial-temporal effects (Table 2a; Figure S2). As drawn by NMDS, many edaphic variables and fungal functions significantly correlated with the local assemblage of fungi (Figure S2). The assemblage of MOTUs within families did mainly vary with the site, interacting with the tree genotype (e.g. Atheliaceae, Russulaceae, Sebacinaceae, Thelephoraceae) and the season (e.g. Amanitaceae, Herpotrichiellaceae, Mortierellaceae, Tricholomataceae), depending on the family (Table S4).

Concerning the regional species pool, total fungal β-diversity was unaffected by the tree genotype or the season, while a strong site effect interacting with the rest of factors was observed (Table 2b; Figure S3). Ascomycetes, Zygomycetes, and saprotrophic fungi were more β-diverse (i.e. more heterogeneous) in autumn than spring, while the ECM guild showed the opposite pattern (Table 2b; Figure S3). Except for ECM, a significant strong site effect was observed for all fungal guilds, generally with lower β-diversity (i.e. more homogeneous) in Cabañeros site (Table 2b; Figure S3).

Table 2 | (a) Assemblage of MOTUs and (b) Beta-diversity of the total fungal community and of representative subgroups: effects of tree genotype (G), season (S), site (Sit) and their interactions assessed by permutation variance analyses and Multivariate Homogeneity of Groups Dispersions, respectively. df = degrees of freedom. F and *p*-value: **p*<0.05; ***p*<0.01;****p*<0.001. ASCO = ascomycetes; BASI = basidiomycetes; ZYGO = zygomycetes; ECM= ectomycorrhizal; SAP = saprotrophic.

		TOTAL		ASCO		BASI		ZYGO		ECM		SAP	
(a) Assemblage	df	R^2	F	R^2	F	R^2	F	R^2	F	R^2	F	R^2	F
Tree genotype	2	0.04	**1.8****	0.04	**2.1****	0.04	**1.7***	0.03	**1.8***	0.04	**1.7***	0.04	**1.9****
Season	1	0.05	**4.5*****	0.07	**6.8*****	0.03	**2.6****	0.19	**21.6*****	0.03	**2.8****	0.12	**11.6*****
Site	2	0.25	**11.8*****	0.26	**13.6*****	0.27	**12.6*****	0.18	**10.2*****	0.28	**13.7*****	0.24	**13.1*****
G x S	2	0.01	0.6	0.01	0.7	0.01	0.4	0.01	0.8	0.01	0.4	0.01	0.8
G x Sit	4	0.06	**1.4****	0.06	**1.5***	0.07	**1.5****	0.04	1.1	0.07	**1.6****	0.05	**1.5***
S x Sit	2	0.03	1.2	0.03	1.4	0.02	0.9	0.05	**2.8****	0.02	1.1	0.03	**1.7***
G x S x Sit	4	0.03	0.6	0.03	0.7	0.02	0.3	0.02	0.6	0.01	0.3	0.03	0.7
(b) β-diversity	df	R^2	F	R^2	F	R^2	F	R^2	F	R^2	F	R^2	F
Tree genotype	2	0.01	0.2	0.04	1.3	0.02	0.7	0.67	0.3	0.00	0.0	0.00	0.0
Season	1	0.00	0.3	0.18	**1.5*****	0.04	3.0	0.37	**40.7*****	0.63	**114*****	0.32	**31.3*****
Site	2	0.50	**33.7*****	0.24	**10.5*****	0.42	**23.8*****	0.11	**4.3***	0.03	1.0	0.12	**4.8****
G x S	5	0.02	0.2	0.24	**4.1****	0.07	0.9	0.35	**6.9*****	0.60	**19.1*****	0.33	**6.4*****
G x Sit	8	0.50	**7.7*****	0.29	**3.1****	0.43	**5.9*****	0.17	1.6	0.03	0.2	0.15	1.3
S x Sit	5	0.47	**11.5*****	0.35	**7.0*****	0.44	**10.2*****	0.52	**14.0*****	0.55	**15.3*****	0.41	**9.1*****
G x S x Sit	17	0.50	**3.1*****	0.45	**2.5****	0.46	**2.6****	0.44	2.4**	0.50	**3.0*****	0.37	1.8

Linking fungal diversity, abiotic and biotic environment, and ecosystem functioning

When modelled as a function of abiotic variables, higher fungal α-diversity was explained by greater values of relative humidity, pH, EC and N, or by lower values of K and C:N (Table 3),

although variations were observed depending on the fungal guild. For example, high organic matter explained low α-diversity of ascomycetes and zygomycetes, and oppositely high α-diversity of basidiomycetes and ECM (Table 3). The α-diversity of basidiomycetes and ectomycorrhizal fungi did not vary with soil pH or C:N ratio, opposite to the rest of fungi. Furthermore, the productivity of trees particularly affected the ECM fungi, for which higher tree productivity explained lower α-diversity (Table 3).

Table 3 | Generalized linear mixed models testing the response of fungal alpha-diversity to edaphic variables or DBH, and to the factors tree genotype and season. The site was included as random factor within models. Interactions were not significant. Degrees of freedom in all models: numDF =1 and denDF = 62. R2adj, t and p values of the α-diversity~edaphic variable models; Significance: . <0.1; * <0.05; ** <0.01; ***<0.001; ns = not significant. RH= relative humidity, EC = electric conductivity, OM = organic matter, N = nitrogen, P = phosphorous, K = potassium, C:N = carbon:nitrogen ratio, DBH = tree diameter at breast height.

	RH (%)	pH	EC (µS/cm)	OM (%)	N (%)	P (mg/kg)	K (mg/kg)	C:N	DBH (cm)
Total MOTUs									
R^2_{adj}	0.55	0.48	0.46	0.44	0.53	0.55	0.52	0.49	0.50
t *p*	2.4***	2.1**	0.2***	-1.0	1.6**	3.2	-0.3*	-3.2***	-1.5
Tree genotype (*p*)	ns	ns	ns	ns	ns	ns	ns	ns	ns
Season (*p*)	ns	***	*	***	***	***	***	***	***
Ascomycetes									
R^2_{adj}	0.73	0.75	0.73	0.74	0.74	0.75	0.73	0.76	0.74
t *p*	0.3***	3.2***	-0.2***	-1.1***	0.8	1.4**	-1.6***	-2.3***	0.8
Tree genotype (*p*)	ns	*	ns	ns	ns	ns	ns	ns	ns
Season (*p*)	ns	ns	ns	ns	ns	ns	ns	ns	ns
Basidiomycetes									
R^2_{adj}	0.25	0.12	0.11	0.20	0.24	0.14	0.14	0.11	0.30
t *p*	2.3	1.5	0.4	1.4*	2.3*	0.9*	0.4	-0.8	-3.4*
Tree genotype (*p*)	*	*	*	ns	ns	*	*	*	**
Season (*p*)	*	ns	ns	ns	ns	ns	ns	ns	ns
Zygomycetes									
R^2_{adj}	0.62	0.62	0.62	0.63	0.62	0.72	0.62	0.67	0.62
t *p*	0.5***	0.3*	-1.0***	-1.7*	0.8**	4.6***	-0.1	-3.0***	0.4
Tree genotype (*p*)	ns	ns	ns	ns	ns	ns	ns	ns	ns
Season (*p*)	ns	*	*	*	*	***	*	ns	*
Ectomycorrhizal									
R^2_{adj}	0.34	0.28	0.27	0.33	0.36	0.27	0.27	0.26	0.55
t *p*	1.7**	0.1	0.4***	1.1*	2.1	1.5***	2.7	-1.1	-4.5*
Tree genotype (*p*)	ns	ns	ns	ns	ns	ns	ns	ns	***
Season (*p*)	*	ns	ns	ns	ns	ns	ns	ns	ns
Saprotrophs									
R^2_{adj}	0.67	0.71	0.67	0.66	0.67	0.67	0.67	0.66	0.66
t *p*	0.1***	2.6***	-0.2***	-0.6	0.2*	2.3***	0.2**	-1.2***	0.2
Tree genotype (*p*)	ns	*	ns	ns	ns	ns	ns	ns	ns
Season (*p*)	ns	ns	ns	ns	ns	*	ns	ns	ns

The tree genotype mainly influenced the glucuronidase activity (Table 4), while most C-cycle related enzymes varied with the season, especially for ascomycetes, zygomycetes and the saprotrophic guild, (Table 4). In most cases, fungal α-diversity significantly explained ecosystem functions related with the degradation of hemicellulose (i.e. xylosidase, glucuronidase) and recalcitrant C compounds (i.e. laccase) (Table 4). For example, the α-diversity of basidiomycetes was negatively related with almost all C-cycle processes (Table 4). Furthermore, high α-diversity of saprotrophs explained high C turnover (Table 4), whereas contrarily high ectomycorrhizal α-diversity explained low C-cycling (Table 4).

The structural-equation model provided a good fit for all enzymatic activities, with non-significant f value (χ^2 = 4.90; P = 0.672) and with goodness-of-fit indices (RMSEA < 0.001, NFI and GFI > 0.97). Significant effects differed depending on the enzymatic set (Figure 3). In all cases, the tree productivity marginally and positively affected the P content in soil, on which pH had a strong negative effect. Edaphic variables had positive (i.e. pH, soil humidity, P) or negative (i.e. OM) effects on overall fungal diversity (Shannon). The productivity of trees exerted a positive and direct effect over cellulose degrading-enzymes and a marginal effect over hemicellulose degrading ones (Figure 3). Hemicellulose degrading-enzymes and laccase activities were positively affected by soil humidity, and laccase also by pH. By contrast, the soil humidity and pH negatively affected N-cycle enzymes. Phosphatase and N-cycle enzymes were significantly more active with increased OM. Total fungal diversity was negatively related with hemicellulose degrading-enzymes and phosphatase activity (Figure 3).

Table 4 | Generalized linear mixed models testing the response of functional traits to fungal alpha-diversity and to the factors tree genotype (G) and season (S). Interactions were not significant. The site was included as random factor within models. Degrees of freedom in all models: numDF =1 and denDF = 62. The t (F) and *p* values correspond to the enzyme~α-diversity relationship. Significance of tree genotype (G) and season (S) *p* = . <0.1 *, <0.05, ** <0.01, ***<0.001, ns = not significant.

	Total MOTUs				**Ascomycetes**				**Basidiomycetes**				**Zygomycetes**				**Ectomycorrhizal**				**Saprotrophs**			
	α-div		G	S	α-div		G	S	α-div		G	S	α-div		G	S	α-div		G	S	α-div		G	S
	R^2_{adj}	t *p*	*p*	*p*	R^2_{adj}	t *p*	*p*	*p*	R^2_{adj}	t *p*	*p*	*p*	R^2_{adj}	t *p*	*p*	*p*	R^2_{adj}	t *p*	*p*	*p*	R^2_{adj}	t *p*	*p*	*p*
Glucosidase	0.35	-0.5	ns	*	0.28	-1.1**	ns	ns	0.38	-2.8*	ns	ns	0.28	0.8	ns	ns	0.43	-2.5**	ns	ns	0.30	0.5	ns	ns
Cellobiohydrolase	0.27	-1.1	ns	*	0.21	-0.8*	ns	ns	0.33	-2.9**	ns	.	0.18	1.6	ns	*	0.37	-2.9**	ns	.	0.21	1.8*	ns	ns
Xylosidase	0.68	2.2***	.	*	0.68	2.0***	.	***	0.68	-0.7**	ns	**	0.69	1.3***	ns	***	0.70	-0.72***	ns	**	0.69	2.1***	ns	***
Glucuronidase	0.38	1.9***	**	ns	0.38	1.3***	*	***	0.39	-0.3**	**	ns	0.40	0.4***	*	*	0.39	-0.2***	**	ns	0.40	1.0***	**	*
Laccase	0.57	2.9***	ns	.	0.55	0.5***	ns	***	0.61	0.7	ns	ns	0.62	2.2***	ns	ns	0.58	0.6***	ns	ns	0.60	0.3***	ns	**
Phosphatase	0.11	-0.5	ns	ns	0.24	0.03	ns	ns	0.21	-1.6	ns	ns	0.26	-1.1	ns	.	0.16	-1.1	ns	ns	0.13	-1.0	ns	ns
Chitinase	0.01	-1.5	ns	ns	0.05	-0.2	ns	ns	0.09	-3.0**	ns	ns	0.02	-0.8	ns	ns	0.06	-2.2*	ns	ns	0.01	0.2	ns	ns
Leucine	0.24	0.2	.	*	0.22	0.7	*	**	0.26	-1.2	ns	**	0.20	0.2*	*	.	0.28	-0.8	.	**	0.27	-0.0	.	**

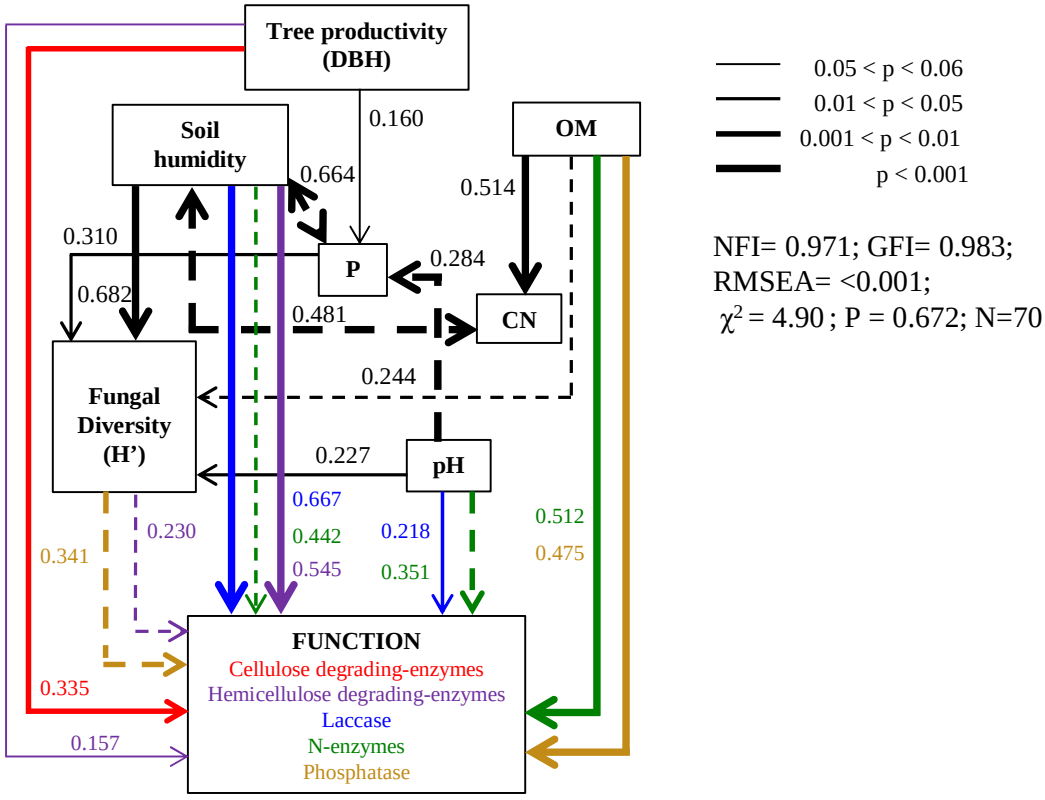

Figure 3 | Path diagrams representing hypothesized causal relationships among the influence of tree productivity, biotic and abiotic predictors and ecosystem functioning. Different colours correspond to different groups of enzymes related with C, N and P cycles. Arrows depict casual relationships: positive effects are indicated by solid lines, and negative effects by dashed lines, with standardized estimated regression weight values (SRW) indicated. Arrow widths are proportional to p values. Paths with coefficients non-significant different from 0 (p>0.08) are omitted. Fit statistics of the model (NFI, GFI and RMSEA) and sample size (N) are given for all proposed models.

DISCUSSION

In addition to recognized abiotic features such as soil moisture, organic matter content, and acidity, our study reveals biotic (i.e. tree genotype) and spatial-temporal (i.e. site, season) factors as key agents structuring fungal communities in Mediterranean forests, and brings out mechanistic patterns linking fungal diversity and environmental conditions with functional traits.

We found high fungal diversity associated with *P. pinaster*, similar to that previously reported for this tree species (Rincón *et al.*, 2014; Buscardo *et al.*, 2015). As predicted, the tree genotype was an important agent structuring the fungal communities associated with *P. pinaster*, mainly through influencing their assemblage and the diversity of certain groups, such as basidiomycetes and representative fungal families. We observed an overrepresentation of basidiomycetes under the Mediterranean and African genotypes respect to Atlantic genotypes, probably in relation with a high representation of ectomycorrhizal fungi within this phylum and/or the quantity/quality of the carbon inputs delivered by trees. Similar to our results, Gehring and Whitham (1991) observed a much higher negative effect of herbivory on ectomycorrhizal fungi under susceptible than resistant pinyon pines and they also detected more diversity of basidiomycetes under the resistant trees, Moreover, fast and low growing spruce clones differing in their nutrients requirements have been shown to associate different ECM fungi both in greenhouse (Velmala *et al.*, 2013) and field plantations (Korkama *et al.*, 2006). In our study, when fungal diversity was responsive to the tree genotype, main differences were found only under one of the two less productive trees i.e. Atlantic, indicating the importance of additional factors as for example the quality of tree organic inputs. However, it should be additionally considered that a single tree may associate multiple fungal genotypes and each interacting organism (i.e. plant-fungus-fungus) can differently respond to the same environmental constraints or/and stimuli (Bahram *et al.*, 2011; Johnson *et al.*, 2012), which greatly complicate interpreting interaction outcomes.

As expectable in a Mediterranean ecosystem with contrasted annual climatic variation, the season exerted a great influence on the structure of fungal communities. Both α and β-diversity of the total fungal community were generally higher in autumn than spring, when peaks of spore dispersion, as well as of mycelium and sporocarp production occur (de la Varga *et al.*, 2013; Boddy *et al.*, 2014). However, when analyzed by life-style, the strategy changed from ectomycorrhizal-dominated communities in spring to saprotrophic ones in autumn, probably in relation with the preference and/or availability of resources (i.e. belowground carbon exudation or litter fall). Together with the season effects, the site was a strong filter at local and regional scales for all fungal guilds, and unequivocal signs of fungal site dependent responses were observed emphasizing the importance of local environment and processes, as recently underlined (Tedersoo

et al., 2016). This spatial-temporal habitat filtering leaded in all cases to more heterogeneous communities probably by increasing the competition of species (Olden *et al.*, 2004; Flores-Rentería *et al.*, 2016). Spatial-temporal scale fungal shifts are tightly linked to the environmental conditions and the phenology of trees, with the light, soil pH, soil nutrients, temperature and moisture as main abiotic drivers (Cooke *et al.*, 1993; Buée *et al.*, 2005; Coince *et al.*, 2014; Rincón *et al.*, 2015), many of them higly related with the assemblage and diversity of fungi in our study.

Are soil ECM fungal communities particularly responsive to the tree host?

The α-diversity of ectomycorrhizal fungi was quite independent of the tree genotype. However, our initial hypothesis that fungal responses to the tree genotype would be particularly affecting obligate biotrophic fungal guilds was partially supported by the response of representative ectomycorrhizal families (i.e. usually less α-diverse under the Atlantic trees), and by β-diversity results (i.e. significant interactions of tree genotype with site and season). A potential host filtering effect was supported by the indicator species associated with each tree genotype that were mainly ectomycorrhizal and more similar between the Mediterranean and African trees. Besides, these results indicated that, in some cases, fungal host preference was dependent on the particular seasonal and site conditions (i.e. environmental filtering). All these findings support that the tree genotype may select their associated fungi, particularly the ectomycorrhizal ones, and that this is likely to be context dependent, suggesting that the plant can modulate its associated microbial community for a dynamical adjustment to the environment (Vandenkoornhuyse *et al.*, 2015). The productivity of trees did not influence total fungal diversity, but it negatively impacted that of ECM fungi, probably indicating a stronger host filtering effect on fungi with which establishing an exchange partnership (Johnson *et al.*, 2010). This could be related with preferential host plant photosyntate allocation to more beneficial (Bever *et al.*, 2009), or less carbon demanding fungi (Gehring *et al.*, 2014) observed within spatially structured mycorrhizal fungal communities. This has been interpreted as a mechanism for mutualism stabilization (Kiers *et al.*, 2011). In our study, together with strong seasonal effects, the tree genotype was implicated in the response of some ecosystem functions to variations in fungal α-diversity (e.g. hemicellulose degradation). In concordance with previous studies (Bending and Read, 1996; Bailey *et al.*, 2005; Velmala *et al.*, 2013; Lamit *et al.*, 2016), altogether our results give evidences to support that the differences on photosynthetic productivity (quantity/quality) of the tree genotypes may be at the origin of their dissimilar structural and functional associated fungal communities, especially the ectomycorrhizal ones. Additionally, the less evident effect of the tree genotype on saprotrophic fungi could probably indicate a greater

dependence of this fungal guild on site and seasonal variations, i.e., temperature and water availability. Different requirements of main fungal guilds, i.e., ectomycorrhizal fungi would need more nitrogen, and saprotrophs more moisture, have been translated into niche differentiation in forest soils (Peay, 2016).

Establishing links between fungal diversity, environment, and functional traits

Our results revealed that relevant ecosystem services involved in C turnover were explained not only by variations in total fungal α-diversity but also in that of specific fungal guilds. Ectomycorrhizal fungal α-diversity was negatively related with most C-cycle processes, while that of saprotrophs displayed a positive relation, according to the divergent life history of these two major fungal guilds, and possibly reflecting competitive interactions (Fernandez and Kennedy, 2016; Martin *et al.*, 2016). Results relating α-diversity and C-cycle activities mirrored a possible predominance of functional guilds within taxonomic ones and vice versa (i.e. basidiomycetes and cellulose-degrading ascomycetes could be mostly ectomycorrhizal, and hemicellulose and cellulose-degrading ascomycetes and zygomycetes mostly saprotrophic), results which would deserve further phylogenetic examination. These findings could also reflect separated main mechanisms (i.e. hydrolytic *vs* oxidative) of saprotrophs and ectomycorrhizal fungi for organic matter decomposition (Shah *et al.*, 2015; Fernandez and Kennedy, 2016).

Structural equation models gave a mechanistic integrative view linking fungal diversity, edaphic conditions and functional traits. The productivity of trees directly influenced the cycling of carbon trough triggering cellulose and hemicellulose degrading enzymes, in agreement with the "priming effect" (i.e. increased carbon inputs stimulate microbial decomposition, Phillips *et al.* (2012)). According to Lindahl *et al.* (2002), this could imply the removal of C with retention of N, as nitrogenous compounds are delivered from complex polyphenolic substrates. This could be supported by the direct and positive relation observed between organic matter and N-related enzymes in our study. Laccase, which degrades recalcitrant compounds, was not related to tree's productivity or organic matter, probably because a more subtle interrelation based on the quality and not the quantity of C inputs occurs, though this would merit further analysis. Tree productivity and soil pH controlled phosphorous availability. Together with nitrogen, phosphorous is usually deficient in Mediterranean soils characterized by fast decomposition and extremely thin litter layers (Sardans *et al.*, 2004), a nutritional limitation that may severely reduce the productivity of trees (Plassard *et al.*, 2011). Phosphorus availability increased fungal diversity, which in turn predicted lower phosphatase activity in soil. Plants can increase C allocation to roots and their mycorrhizal associates to alleviate P deficiency (van der Heijden, 2001; Kiers *et al.*, 2011), although the reduced phosphatase activity and its activation by organic matter suggest that

other mechanisms could be operating, e.g. the production of organic acids or chelators, and/or bacterial inputs (Plassard and Dell, 2010; Clarholm *et al.*, 2015). Contrarily to P, high organic matter explained reduced fungal diversity, which in turn predicted higher hemicellulose degrading activity pointing out to the possible dominance of certain fungi more competitive under these conditions.

In conclusion, our results show that the intricate relations between aboveground tree individuals and spatial-temporal variants drive structural shifts in fungal communities with functional consequences that affect relevant ecosystem processes i.e. C turnover, phosphorous mobilization. According to Bardgett *et al.* (2005), we highlight the need of experimental field designs recovering spatial and temporal variability for better predicting the consequences of tree-soil feedbacks. Our results suggest that the tree genotype is able to modulate its associated fungal community to adapt better to the environment by selecting certain fungal consortia, which may influence the functioning of the entire ecosystem.

SUPPORTING INFORMATION

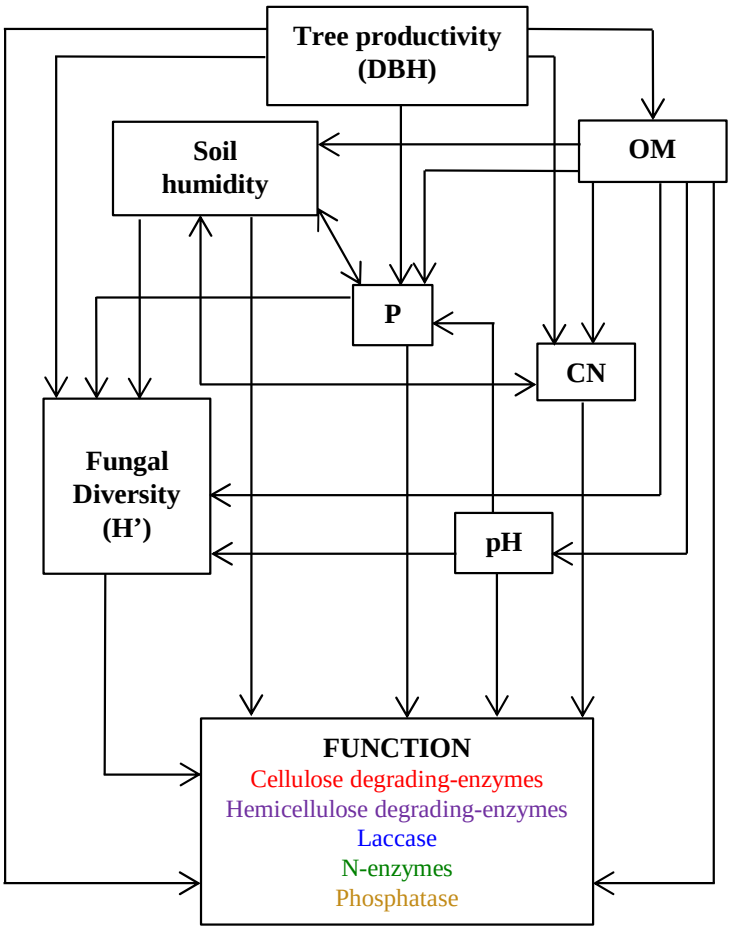

Figure S1 | Proposed path diagram representing hypothesized causal relationships among the influence of tree productivity, biotic and abiotic predictors and ecosystem functioning. Arrows depict casual relationships.

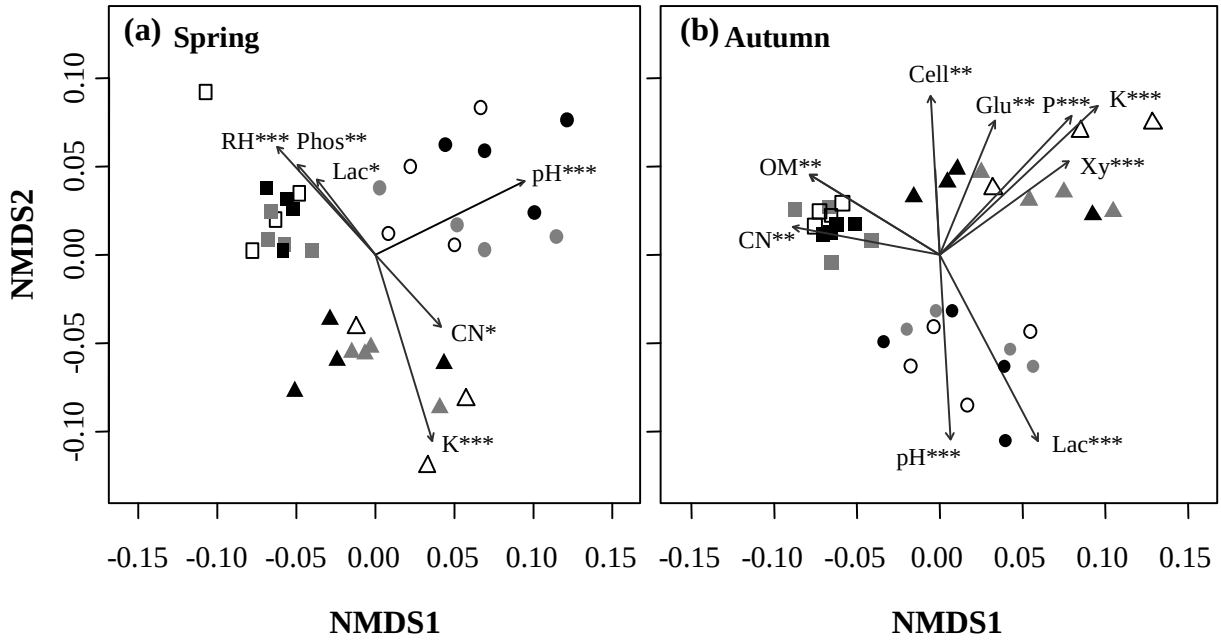

Figure S2 | Assemblage of fungal communities in (a) spring (k=2; stress = 0.16; R^2=0.97), and (b) autumn (k=2; stress = 0.12; R^2=0.99), by tree genotype (black = Atlantic; grey = Mediterranean; white = African) and site (square = Cabañeros-CAB; circle = Riofrío-RIO; triangle = Espinoso del Rey-ESP), analysed by nonmetric multidimensional scaling (NMDS). Vectors represent the strength/direction of the weight of variables (RH = relative humidity; EC = electric conductivity; K = potassium; P = phosphate; OM = organic matter; N = nitrogen; C:N = carbon/nitrogen ratio; DBH = tree diameter ; Glu = glucosidase; Cell = cellobiohydrolase; Xy = xylosidase; Glucu = glucuronidase; Lac = laccase; Phos = phosphatase; Chi = Chitinase; Leu = leucine), on the distribution of fungal MOTUs (*p<0.05; **p <0.01; ***p<0.001).

Figure S3 | Beta-diversity of the total community and representative fungal subgroups associated with (a) different *Pinus pinaster* Ait. genotypes (black = Atlantic; grey = Mediterranean, and white = African), and (b) at different seasons (black = spring, and white = autumn). Boxes represent the interquartile range (IQR) between first and third quartiles and the horizontal line inside represents the median. Whiskers denote the lowest and highest values within 1.5 x IQR from the first and third quartiles, respectively.

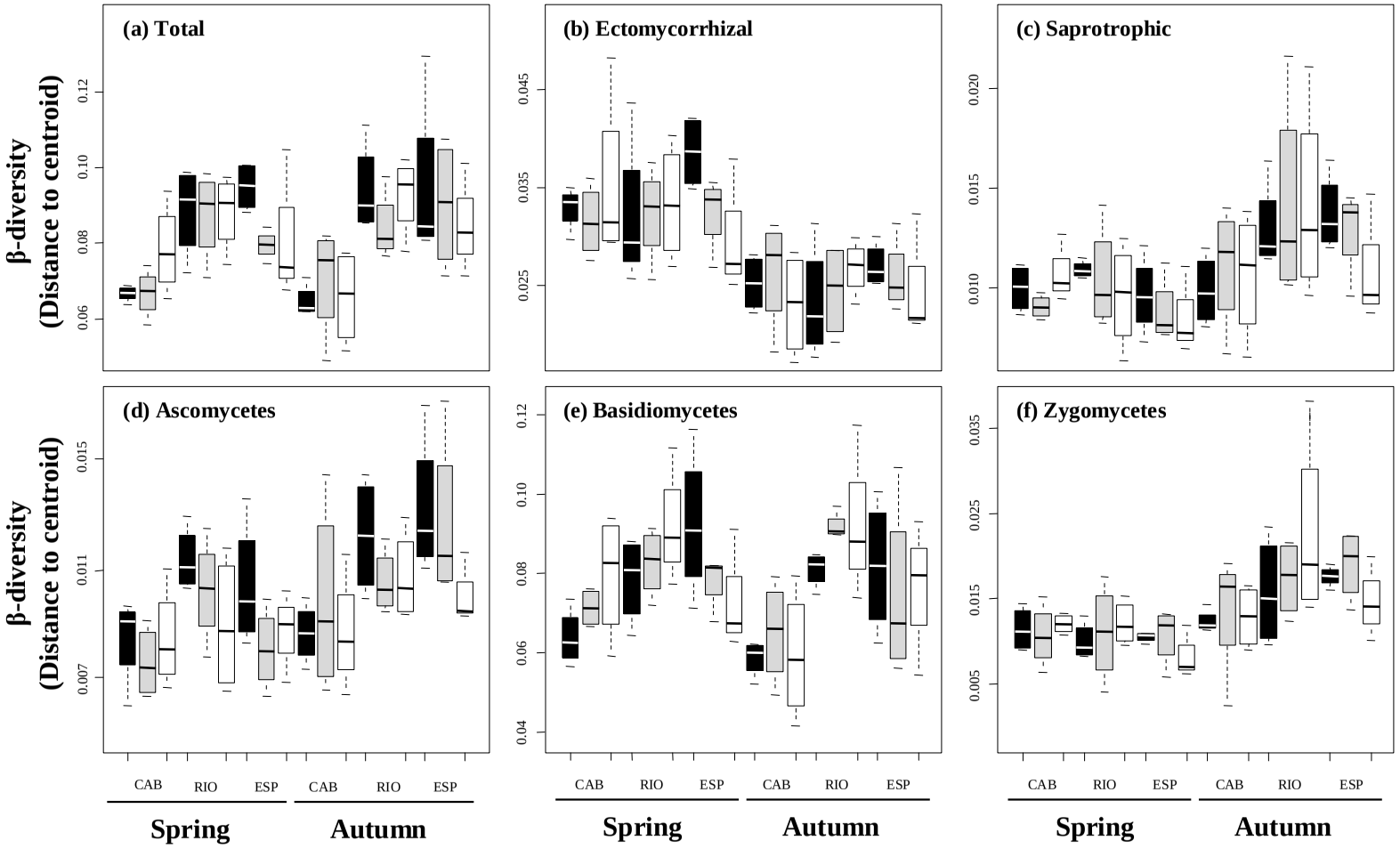

Table S1 | Summary of sequencing and number of MOTUs: (A) total, (B) per tree genotype and season, (C) per site and season and (D) per genotype, site and season. Tree genotype: Atl = Atlantic; Med = Mediterranean; Afr = African. Site: CAB = Cabañeros; RIO = Riofrío; ESP = Espinoso del Rey. SD = standard deviation. * in a per sample basis.

(A) Total

TOTAL	Sequences	MOTUs
Total	166927	1412
mean ± SD	2384 ± 402	191 ± 34

Per tree genotype	Nb. Reads	MOTUs
Atlantic	57982	1065
Mediterranean	56702	1043
African	52243	1042
Atl (mean ± SD)*	2416 ± 407	189 ± 31
Med (mean ± SD)	2362 ± 461	190 ± 35
Afr (mean ± SD)	2375 ± 338	195 ± 36

Per season	Nb. Reads	MOTUs
Spring	93859	1146
Autumn	73068	1193
Spring (mean ± SD)	2682 ± 263	174 ± 23
Autumn (mean ± SD)	2088 ± 277	208 ± 34

Per site	Nb. Reads	MOTUs
Cabañeros	60669	797
Riofrío	55706	953
Espinoso del Rey	50552	912
CAB (mean ± SD)	2528 ± 353	174 ± 24
RIO (mean ± SD)	2321 ± 474	201 ± 32
ESP (mean ± SD)	2298 ± 334	198 ± 38

(B) Per tree genotype and season

	Spring		Autumn	
Per tree genotype	Nb. Reads	MOTUs	Nb. Reads	MOTUs
Atlantic	32541	797	25441	839
Mediterranean	32372	736	24330	835
African	28946	740	23297	841
Atl (mean ± SD)	2712 ± 233	176 ± 25	2120 ± 319	202 ± 32
Med (mean ± SD)	2698 ± 324	169 ± 20	2028 ± 307	210 ± 34
Afr (mean ± SD)	2631 ± 236	177 ± 26	2118 ± 198	212 ± 38

(C) Per site and season

	Spring		Autumn	
Per Site	Nb. Reads	MOTUs	Nb. Reads	MOTUs
Cabañeros	33372	605	27297	634
Riofrío	32191	697	23515	763
Espinoso	28296	628	22256	754
CAB (mean ± SD)	2781 ± 272	170 ± 25	2275 ± 215	179 ± 22
RIO (mean ± SD)	2683 ± 313	181 ± 27	1960 ± 297	221 ± 24
ESP (mean ± SD)	2572 ± 147	170 ± 16	2023 ± 218	226 ± 34

D) Per tree genotype, site and season

	Spring		Autumn	
Cabañeros	Nb. Reads	MOTUs	Nb. Reads	MOTUs
Atlantic	10675	672	9529	714
Mediterranean	11418	656	9187	738
African	11297	714	8581	691
Atl (mean ± SD)	2664 ± 258	168 ± 16	2382 ± 192	179 ± 4
Med (mean ± SD)	2855 ± 372	164 ± 17	2297 ± 196	185 ± 41
Afr (mean ± SD)	2824 ± 195	179 ± 40	2145 ± 237	173 ± 3
Riofrío	Nb. Reads	MOTUs	Nb. Reads	MOTUs
Atlantic	11329	818	7874	904
Mediterranean	10748	681	7343	857
African	10114	676	8298	893
Atl (mean ± SD)	2832 ± 276	205 ± 12	1969 ± 356	226 ± 36
Med (mean ± SD)	2687 ± 418	170 ± 32	1836 ± 307	214 ± 20
Afr (mean ± SD)	2529 ± 214	169 ± 19	2075 ± 251	223 ± 18
Espinoso del Rey	Nb. Reads	MOTUs	Nb. Reads	MOTUs
Atlantic	10555	621	8038	808
Mediterranean	10206	695	7800	923
African	7535	559	6418	753
Atl (mean ± SD)	2639 ± 164	155 ± 11	2010 ± 267	202 ± 30
Med (mean ± SD)	2552 ± 103	174 ± 10	1950 ± 242	231 ± 28
Afr (mean ± SD)	2513 ± 190	186 ± 10	2139 ± 106	251 ± 31

Table S2 | The 20-most abundant fungal MOTUs found in Pinus pinaster Ait. forests under different tree genotypes: Atl = Atlantic. Med = Mediterranean. Afr = African, and at different seasons: Sp = spring. Au = autumn. *= not in the top-20 list of the respective treatment. ECM = ectomycorrhizal; SAP = saprotrophic. ¥ = number of reads.

Tentative	Life	NCBI / UNITE / RDP	BLAST ID	% id	E-value	Total	Genotype¥			Season¥	
							Atl	Med	Afr	Sp	Au
Russula amethystina	ECM	UDB000303	*R. amethystina*	100	1.00E-84	8413	4072	2184	2157	5492	2921
Amphinema sp.	ECM	SH210842	*Amphinema*	100	5.00E-85	7874	3259	2699	1916	5645	2229
Mortierella sp.	SAP	SH214832	*Mortierella*	100	8.00E-69	6232	2327	2074	1831	1443	4789
Tylospora sp.	ECM	FJ013075	*Tylospora*	100	4.00E-79	5780	1637	1932	2211	3369	2411
Russula cessans	ECM	UDB015971	*R. cessans*	100	1.00E-92	4739	764	2130	1845	3437	1302
Hydnellum ferrugineum	ECM	KC571730	*H. ferrugineum*	89	3.00E-77	4506	232*	2138	2136	2351	2155
Sebacina sp.	ECM	SH231619	*Sebacina*	100	3.00E-82	3845	829	1267	1749	2648	1197
Russula torulosa	ECM	UDB011110	*R.torulosa*	100	4.00E-93	3315	1259	1392	664	2593	722*
Mortierella sp.	SAP	DQ093726	*Mortierella*	100	5.00E-83	3069	1061	1091	917	1228	1841
Cenococcum geophilum	ECM	KC967408	*C. geophilum*	98	2.00E-60	2825	1323	788	714	1752	1073
Amphinema sp.	ECM	SH210842	*Amphinema*	99	3.00E-81	2675	1295	784	596	2115	560*
Russula amethystina	ECM	KF850402	*R. amethystina*	98	2.00E-73	2487	868	1195	424*	1291	1196
Clavulina sp.	ECM	SH220805	*Clavulina*	100	3.00E-102	2225	319*	1099	807	1180	1045
Inocybe sp.	ECM	SH231190	*Inocybe*	100	2.00E-77	2084	1218	234*	632	1469	615*
Inocybe posterula	ECM	JF908152	*I. posterula*	99	3.00E-121	1847	212*	1097	538*	1421	426*
Inocybe sp.	ECM	JF908227	*Inocybe*	99	4.00E-111	1764	331*	858	575	813*	951
Tricholoma portentosum	ECM	UDB017949	*T. portentosum*	100	1.00E-120	1691	410*	446*	835	1681	10*
Inocybe mixtilis	ECM	JX679372	*I. mixtilis*	100	2.00E-97	1682	515*	291*	876	922*	760
Russula versicolor	ECM	SH224391	*R. versicolor*	99	2.00E-91	1656	329*	411*	916	1007	649*
Cortinariaceae sp.	ECM	GQ159878	*Cortinarius*	96	4.00E-69	1622	609	642	642*	960	662*

Table S3 | Indicator fungal species at a significant level of $p \leq 0.05$ and their abundances (number of reads) by (A) tree genotype (Atl = Atlantic, Med = Mediterranean, Afr = African), (B) season (spring, autumn), and (C) site (Cab = Cabañeros; Rio = Riofrío; Esp = Espinoso del Rey), and their interactions: (D) tree genotype x season, (E) tree genotype x site and (F) season x site.

(A) Tree Genotype		Reads		
	p	Atl	Med	Afr
Atlantic				
Chaetothyriales sp.	0.022	76	13	12
Cortinarius sp.	0.046	385	4	2
Inocybe sp.	0.012	116	18	0
Basidiomycetes	0.03	96	21	11
Mediterranean				
Tylospora sp	0.019	2	3	108
Atlantic-Mediterranean				
Tomentella sp.	0.02	776	488	25
Mediterranean-African				
Cortinarius sp	0.015	0	125	22
Hydnellum ferrugineum	0.03	232	2133	2104
Hydnellum ferrugineum	0.05	38	911	446
Terfezia leptoderma	0.008	9	70	49
Trechispora sp	0.041	25	93	162
Tylospora sp	0.001	20	168	166

(B) Season		Reads	
	p	Sp	Au
Spring			
Amanita sp.	0.006	181	6
Hydnum sp.	0.029	216	12
Hygrophorus hypothejus	0.04	1307	103
Rhizopogon sardous	0.002	307	15
Sistotrema sp.	0.015	940	36
Tricholoma equestre	0.001	633	20
Tricholoma populinum	0.001	100	3
Tricholoma portentosum	0.003	1681	10
Autumn			
Agaricomycetes sp.	0.004	22	99
Archaeorhizomyces	0.001	75	415
Capnodiales sp.	0.006	18	132
Cortinarius sierraensis	0.028	0	110
Mortierela lignicola	0.001	11	234
Thelephoraceae sp.	0.014	173	824
Trichoderma pubescens	0.001	18	149

(C) Site		Reads		
	p	Cab	Rio	Esp
Cabañeros				
Clavulina sp.	0.001	2215	7	3
Amanita sp.	0.001	187	0	0
Cladophialophora sp.	0.001	187	7	3
Clavulina amazonensis	0.001	470	0	0
C. diasemospermus	0.001	671	16	28
Cryptococcus sp.	0.001	271	21	14
Hydnellum ferrugineum	0.001	4467	7	32
Hydnellum ferrugineum	0.001	1373	2	20
Hydnum sp.	0.001	226	2	0
Inocybe mixtilis	0.002	110	0	0
Inocybe praetervisa	0.001	236	55	1
Inocybe praetervisa	0.001	217	50	0
Inocybe sp.	0.001	565	41	1
Inocybe sp.	0.001	802	0	6
Lyophyllum semitale	0.002	106	0	23
Mortierella humilis	0.004	427	99	88
Mortierella sp.	0.001	268	47	10
Helotiales sp.	0.001	128	18	11
Penicillium sp.	0.002	95	1	21
Phellodon sp.	0.038	104	2	0
Sistotrema sp.	0.001	703	0	1
Sistrotema sp.	0.001	896	66	14
Thelephoraceae sp.	0.016	1022	259	133
Thelephoraceae sp.	0.002	139	1	0
Tricholoma portentosum	0.011	1384	9	298
Riofrío				
Archaeorhizomycetes sp.	0.001	3	323	9
Clavulina sp.	0.004	0	476	15
Clitocybe sp.	0.011	3	145	7
Cortinariaceae sp.	0.002	12	609	274
Hygrophorus hypothejus	0.001	28	1271	111
Inocybaceae sp.	0.003	0	95	8
Inocybe calospora	0.001	0	291	1
Inocybe lacera	0.001	81	203	1
Inocybe mixtilis	0.017	3	1546	133
Inocybe sp.	0.002	1	133	0
MOTU-150	0.003	0	148	2
MOTU-222	0.001	1	105	0
Rhizopogon sardous	0.001	19	272	31
Russula cessans	0.001	32	4675	32

		Reads		
	p	Cab	Rio	Esp
Riofrío				
Russula olivobrunnea	0.001	1	303	112
Russula torulosa	0.001	5	2672	638
Russulaceae sp.	0.022	0	126	1
Thelephoraceae sp.	0.001	2	410	26
Thelephoraceae sp.	0.01	2	775	3
Thelephoraceae sp.	0.024	0	126	0
Tomentella sp.	0.001	16	407	71
Tomentella sp.	0.001	0	120	13
Trechispora sp.	0.001	17	224	39
Espinoso del Rey				
Capnodiales sp.	0.001	2	10	138
Chaetothyriales sp	0.001	16	8	77
Cladophialophora sp.	0.001	4	3	145
Cortinarius sp.	0.014	1	47	343
Helotiales sp.	0.001	10	0	93
Inocybe sp.	0.001	83	8	739
Inocybe subnudipes	0.001	1	301	616
Laccaria laccata	0.001	0	0	131
Laccaria laccata	0.001	0	0	184
Lactarius glaucescens	0.001	0	0	119
MOTU-226	0.001	24	3	76
Pezizaceae sp.	0.002	0	10	114
Piloderma sp.	0.001	1	3	1424
Pseudotomentella atrofusca	0.042	1	42	75
Russula heterophylla	0.002	0	0	296
Russula lepida	0.034	0	0	135
Russula vesca	0.001	0	1	184
Russulaceae sp.	0.001	0	0	518
Russulaceae sp.	0.019	0	1	529
Sebacina sp.	0.001	0	1	119
Sebacinaceae sp.	0.001	0	0	105
Sebacinaceae sp.	0.001	0	0	150
Sebacinaceae sp.	0.001	0	0	137
Sebacinaceae sp.	0.001	0	0	146
Terfezia leptoderma	0.001	0	0	126
Terfezia leptoderma	0.001	6	28	94
Terfezia leptoderma	0.005	24	8	93
Thelephoraceae	0.003	20	25	251
Tomentella coerulea	0.001	72	0	207
Tomentella sp.	0.001	13	46	1230

Continuation **Table S3**

(D) Tree Genotype × Season	
	p
Atl-Med (Spring)	
Tricholoma equestre	0.003
Atl (Spring) + Atl-Med (Autumn)	
MOTU_140	0.005
Med-Afr (Spring & Autumn)	
Tylospora sp.	0.01
Med (Spring) + Atl-Med-Afr (Autumn)	
Capnodiales	0.027
Atl-Med (Spring) + Atl-Med-Afr (Autumn)	
MOTU-643	0.001
Med-Afr (Spring) + Atl-Med-Afr (Autumn)	
MOTU-53	0.004

(E) Tree Genotype × Site	
	p
Atl (Rio)	
Inocybe sp.	0.001
Med (Rio)	
Thelephoraceae	0.006
Atl (Esp)	
Chaetothyriales	0.001
Med (Esp)	
Sebacina sp.	0.001
Sebacinaceae	0.01
Pezizazeae	0.036
Afr (Esp)	
Russula heterophylla	0.007
Atl-Afr (Cab)	
Inocybe sp.	0.001
Sistotrema sp.	0.001
Inocybe mixtilis	0.001
Med-Afr (Cab)	
Hydnellum ferrugineum	0.001
Hydnellum ferrugineum	0.002
Clavulina amazonensis	0.001
Atl-Med (Rio)	
Inocybaceae	0.023
Atl-Afr (Rio)	
MOTU_222	0.001
MOTU_150	0.035
Med-Afr (Rio)	
Inocybe calospora	0.006
Atl-Med (Esp)	
Tomentella sp.	0.001
Russula vesca	0.002
Sebacinaceae	0.001
Laccaria laccata	0.002
Lactarius glaucescens	0.038
Atl-Afr (Esp)	
Piloderma sp.	0.001
Med-Afr (Esp)	
Terfezia leptoderma	0.001
Sebacinaceae	0.001
Russulaceae	0.032

(F) Season × Site	
	p
Spring (Cab)	
Amanita sp.	0.001
Sistotrema sp.	0.001
Hydnum sp.	0.001
Tricholoma portentosum	0.001
Inocybe mixtilis	0.001
Thelephoraceae	0.011
Spring (Rio)	
Rhizopogon sardous	0.001
Tomentella sp.	0.001
Inocybaceae	0.015
Clavulina sp.	0.007
Spring (Esp)	
Lactarius glaucescens	0.001
Russulaceae	0.003
Laccaria laccata	0.004
MOTU-94	0.006
Autumn (Rio)	
Clitocybe sp.	0.043
Autumn (Esp)	
Capnodiales	0.001
Spring (Cab-Rio)	
Tricholoma populinum	0.001
Autumn (Cab-Rio)	
Agaricomycetes	0.001
Spring (Cab-Rio) + Autumn (Rio)	
Cortinariaceae	0.001
Inocybe lacera	0.001
Spring (Cab-Esp) + Autumn (Cab)	
Rhodocybe gemina	0.008
Rhizopogon luteolus	0.032
Spring (Cab-Esp) + Autumn (Esp)	
Terfezia leptoderma	0.008
Russula densifolia	0.032
Spring (Cab) + Autumn (Cab+Rio)	
Cryptococcus sp.	0.008
Mortierella sp.	0.032
Spring (Rio-Esp) + Autumn (Rio)	
Russula torulosa	0.001
Cortinarius sp.	0.001
Cortinariaceae	0.002
Russula olivobrunnea	0.001
Inocybe cincinnata	0.001
Inocybe geophylla	0.003
Clavaria sp.	0.004
Inocybe mixtilis	0.044
Spring (Rio-Esp) + Autumn (Esp)	
Inocybe lilacina	0.001
Inocybe sororia	0.001
Inocybe posterula	0.001
Spring (Cab-Esp) + Autumn (Cab+Esp)	
Helotiales	0.001
Hamigera insecticola	0.001
Sebacina sp.	0.006
Russula amethystina	0.001
Amphinema sp.	0.006
Russula amethystina	0.001
Spring (Cab) + Autumn (Cab+Rio+Esp)	
Mortierella humilis	0.001
Spring (Rio-Esp) + Autumn (Rio+Esp)	
Exophiala equina	0.001
Cladophialophora	0.001
Cenococcum geophilum	0.001
Tomentella sp.	0.001
Cladophialophora	0.001
Lecanorales	0.001
Inocybe sp.	0.001
Penicillium restrictum	0.003
Ascomycota	0.001
Tomentella sp.	0.001
Inocybaceae	0.001
Thelephoraceae	0.001

Table S4 | Fungal community assemblage of representative fungal families by tree genotype (G), season (S), site (Sit) and their interactions evaluated by permutation variance analyses (ADONIS). df = degrees of freedom. Significance level: *p<0.05; **p<0.01;***p<0.001). § = ectomycorrhizal families.

		Tree genotype	*Season*	*Site*	*G x S*	*G x Sit*	*S x Sit*	*G x S x Sit*
	df	*2*	*1*	*2*	*2*	*4*	*2*	*4*
Amanitaceae§	R^2	0.04	0.01	0.19	0.03	0.02	0.15	0.01
	F	2.02	1.37	**9.39*** **	1.27	0.45	**7.19*** **	0.36
Atheliaceae	R^2	0.05	0.06	0.15	0.01	0.08	0.04	0.02
	F	**2.11****	**4.95*** **	**6.31*** **	0.46	**1.74****	1.77	0.34
Archaeorhizomycetaceae	R^2	0.05	0.13	0.02	0.03	0.13	0.01	0.1
	F	**2.25***	**11.98*** **	0.95	1.31	**3.00****	0.48	**2.39***
Bankeraceae§	R^2	0.05	0.00	0.21	0.00	0.08	0.00	0.00
	F	2.04	0.09	**8.20*** **	0.07	1.69	0.10	0.03
Clavulinaceae§	R^2	0.03	0.01	0.31	0.01	0.05	0.02	0.02
	F	1.27	0.99	**14.55*** **	0.33	1.19	1.07	0.44
Cortinariaceae§	R^2	0.03	0.02	0.18	0.02	0.05	0.03	0.04
	F	1.18	1.6	**7.28*** **	0.62	0.95	1.19	0.72
Entolomataceae	R^2	0.00	0.02	0.11	0.01	0.05	0.06	0.03
	F	0.15	1.76	**4.23****	0.55	0.97	2.18	0.58
Herpotrichiellaceae	R^2	0.05	0.05	0.33	0.02	0.05	0.05	0.03
	F	**3.28****	**6.17*** **	**20.43*** **	1.1	1.44	**2.79****	0.97
Hygrophoraceae	R^2	0.05	0.07	0.16	0.05	0.04	0.08	0.05
	F	**2.61***	**7.41****	**8.41*** **	**2.83***	1.16	**4.46****	1.31
Hypocreaceae	R^2	0.01	0.12	0.07	0.01	0.08	0.03	0.05
	F	0.49	**10.39*** **	**2.76***	0.61	1.62	1.30	1.10
Inocybeaceae§	R^2	0.03	0.00	0.25	0.01	0.05	0.02	0.02
	F	1.31	0.22	**10.39*** **	0.58	0.99	0.72	0.42
Mortierellaceae	R^2	0.03	0.23	0.18	0.01	0.05	0.05	0.02
	F	**1.8***	**27.99*** **	**10.91*** **	0.64	1.46	**3.12****	0.68
Pezizaceae	R^2	0.05	0.01	0.14	0.01	0.06	0.02	0.02
	F	**2.09***	0.52	**5.48*** **	0.42	1.17	0.59	0.44
Pyronemataceae	R^2	0.06	0.05	0.11	0.02	0.03	0.04	0.03
	F	**2.21***	**4.22****	**4.22*** **	0.85	0.68	1.53	0.67
Rhizopogonaceae§	R^2	0.04	0.04	0.12	0.02	0.03	0.10	0.05
	F	1.81	**3.84***	**5.45*** **	0.69	0.74	4.22	1.03
Russulaceae§	R^2	0.02	0.00	0.28	0.01	0.09	0.03	0.02
	F	1.1	0.21	**13.24*** **	0.62	**2.16****	1.2	0.41
Sebacinaceae§	R^2	0.03	0.01	0.21	0.002	0.1	0.02	0.02
	F	1.42	1.21	**8.78*** **	0.08	**2.07****	0.96	0.41
Telephoraceae§	R^2	0.04	0.02	0.19	0.01	0.08	0.01	0.01
	F	**1.59***	1.32	**7.66*** **	0.43	**1.66****	0.59	0.22
Trichocomataceae	R^2	0.03	0.07	0.27	0.01	0.06	0.02	0.03
	F	1.36	**7.44*** **	**13.72*** **	0.57	1.52	0.91	0.87
Tricholomataceae	R^2	0.02	0.08	0.10	0.01	0.06	0.05	0.04
	F	0.77	**6.53*** **	**3.89*** **	0.54	1.16	**2.19***	0.77
Tuberaceae§	R^2	0.05	0.02	0.18	0.00	0.02	0.08	0.01
	F	2.03	2.02	**7.50*** **	0.14	0.47	**3.35***	0.27
Umbelopsidaceae	R^2	0.03	0.08	0.16	0.02	0.03	0.04	0.02
	F	1.4	**6.81*** **	**6.76*** **	0.63	0.53	1.71	0.36

Chapter 3

Plant genotype modulates nutrient cycling through its belowground microbial cloud

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INTRODUCTION

Ecosystem engineers are species capable to structure communities, altering the conditions for the associated biota and modifying the ecosystem functions (Ellison *et al.*, 2005). Since the emergence of community genetics, ecologists have also described how the community structure and ecosystem functioning can be mediated by some particular genotypes within a single species (Vellend and Geber, 2005). Specially in species-poor ecosystems, the genetic diversity of a particular species may have critical ecological consequences at the community and ecosystem levels, which can be even comparable in importance to the effects of species diversity (McGraw, 1995; Hughes *et al.*, 2008). These ecological consequences include the modification of the network of interactions among species (Whitham *et al.*, 2003). Indeed, a high number of studies have reported that intraspecific diversity can influence the structure of communities and the ecosystem functioning in plant-plant and plant-animal interactions (Gamfeldt *et al.*, 2005; Wimp *et al.*, 2005; Pakeman *et al.*, 2006; Whitham *et al.*, 2006; Crutsinger *et al.*, 2008).

The plant genotype can determine the composition of plant-associated microbial communities, as has been shown for both ectomycorrhizal (ECM) fungi and soil bacteria (Gehring and Whitham, 1991; Korkama *et al.*, 2006; Schweitzer *et al.*, 2008; Peiffer *et al.*, 2013; van der Heijden and Schlaeppi, 2015). This can be attributed to the differences both in plant growth performance and in the carbon supplied to the soil through litter or root exudates (Priha *et al.*, 1999; Korkama *et al.*, 2006; van der Heijden and Schlaeppi, 2015). Microbes, particularly heterotrophic soil fungi and bacteria, perform essential ecosystem functions such as litter decomposition and organic matter mineralization, mainly through the production of a wide set of extracellular enzymes (Baldrian, 2014). Thus, it is expected that the plant genotype, by determining the structure of its microbial partners, can exert cascading effects on the performance of ecosystems related to nutrient cycling. The relationship between the structure of ecological communities and the ecosystem functioning can be better predicted by using phylogenetically-informed metrics of community structure (Cadotte *et al.*, 2008; Navarro-Cano *et al.*, 2014; Pérez-Valera *et al.*, 2015). This observation responds to the fact that phylogeny provides an integrated measure of all functional traits, since trait similarity is usually determined by common ancestry (Pausas and Verdú, 2010; Goberna and Verdú, 2016).

The effects of the plant genotype on the belowground microbial communities can be ideally studied through the symbiosis between plants and ECM fungi. ECM fungi depend on the carbon supplied by plants and, in turn, improve the uptake from soil of limiting nutrients, predominantly N and P, for the host plant (Smith and Read, 2008). The plant creates a flux of carbohydrates towards the roots to maintain the symbiosis, creating an extremely rich environment where numerous microorganisms, such as bacteria, proliferate (Rincón *et al.*, 2005;

Frey-Klett *et al.*, 2007). This can be seen as a microbial cloud, whose community structure is influenced by the physical and chemical properties of this microhabitat, some of which are determined by the genetics of the host plant (Peiffer *et al.*, 2013). As plant rhizodeposits are the key energy supply for the rhizospheric microbiota (Lynch and de Leij, 2001), the plant modulates these inhabitants by excreting selective exudate cocktails (Hartmann *et al.*, 2009; Steinauer *et al.*, 2016). These observations led us to hypothesize that the genotype of the tree host leaves a phylogenetic signature in the microbial communities by favouring particular microbial clades thriving in the rhizosphere (Figure 1). In turn, the phylogenetic structure of microbial communities modulates important ecosystem functions like those related to nutrient cycles (Figure 1).

To test the hypothesis that nutrient cycling is modulated by the plant genotype through its belowground microbial cloud, we studied the bacterial and ECM fungal communities associated with different genotypes of *Pinus pinaster*. A clear genetic differentiation exists among trees coming from the three main geographic provenances, hereafter referred as Atlantic, Mediterranean and African (Alía and Moro, 1996; González-Martínez *et al.*, 2004; Rodríguez-Quilón *et al.*, 2016). Differences across genotypes are further reflected in the plant phenotype, in terms of stem shape, growth and biomass, pest resistance as well as frost and drought tolerance (Alía and Moro, 1996; González-Martínez *et al.*, 2004). We analysed trees from all three genotypes that had been experimentally planted in three replicated long-term common garden experiments. The use of these replicated common gardens allowed us to tease apart the effect of the plant genotype from that of the planting site. Our objective was to study whether i) the genotype of *P. pinaster* determines the phylogenetic community structure of symbiotic ECM fungi and rhizospheric bacteria regardless of the environmental (i.e. climatic and edaphic) conditions, and ii) the differential phylogenetic structure of the soil microbial community is further reflected in the ecosystem functioning measured as the rates of carbon, nitrogen and phosphorous cycling.

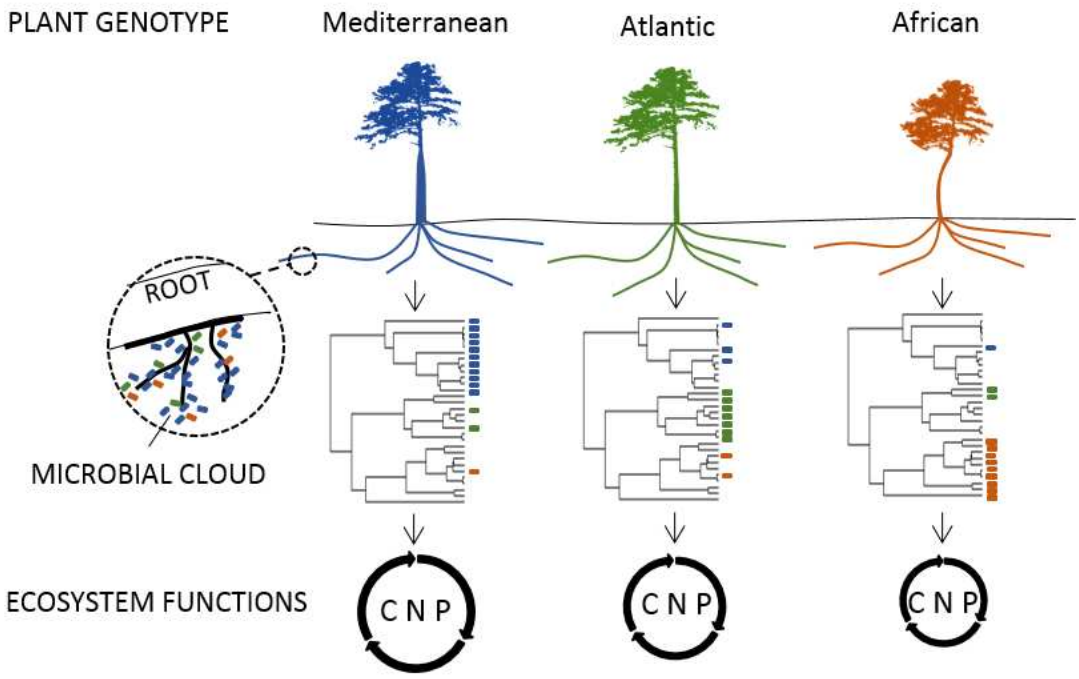

Figure 1 | Intraspecific variations in the plant genotype might determine the phylogenetic community structure of the rhizospheric microbial cloud that further modulates the rates of ecosystem functions related to nutrient cycling. Plant genotypes, which are depicted in different colours, have distinct phenotypes (e.g. biomass production) that may lead to differential resource allocation to their symbiotic ECM fungi and/or exudation to the rhizosphere. This may overrepresent specific microbial lineages that in turn differ in their productivity in terms of enzymatic breakdown of C, N and P organic substrates. Pine silhouettes have been slightly modified from that downloaded from http://www.phylopic.org (MM Tobias) to reflect different pine genotypes. The original image is licensed under a Creative Commons 3.0 license (http://creativecommons.org/licenses/by/3.0).

MATERIAL AND METHODS

Study sites and sample collection

The study was conducted in three ~45-year-old common gardens of *P. pinaster* established with trees from several geographic origins (Alía and Moro, 1996). The three planting sites were located in central Spain in Cabañeros (39º 22'N, 4º 24'W), Riofrío (39º 8'N, 4º 32'W) and Espinoso del Rey (39º 36'N, 4º 48'W). Soil characteristics and climatic features of sites are summarized in Table S1 in Supporting Information.

Plantations in all sites had a completely randomized block design with four blocks, each one including 16 *P. pinaster* individuals from several geographic origins planted 2.5 m apart (Alía and Moro, 1996). Here, we studied each *P. pinaster* provenance, which have been previously shown to be genotypically and phenotypically distinct (Alía and Moro, 1996; González-Martínez *et al.*, 2004; Rodríguez-Quilón *et al.*, 2016), by analysing trees coming from the following geographic origins: Atlantic (Galicia, NW-Spain), Mediterranean (Valencia, E-Spain) and African (Jbel

Tassali, Morocco). Tree genotypes differed in their productivity in terms of biomass, the Mediterranean genotype showing the highest diameter at 1.30 m (DBH) (Mediterranean = 28.9 cm ± 1.0 a; Atlantic = 22.6 cm ± 1.7 b; African = 20.3 cm ± 0.9 b; $F_{2,26}$ =13.9, P<0.001). Differences in DBH associated with the plant genotypes were consistent across planting sites (site × genotype interaction: $F_{4,26}$ =1.65, P>0.1). We selected three trees per genotype and experimental block, making a total of 108 trees (3 sites × 3 plant genotypes × 4 blocks × 3 trees). However, due to the former opening of a firebreak in one of the sites (Espinoso del Rey), 6 trees were lacking and finally 102 trees were sampled.

Soil sampling was performed during the growing season in spring 2012, when canopy carbon is amply drained to roots, leading to high bacterial (Bardgett *et al.*, 2005) and fungal activity (Kaiser *et al.*, 2010). Four samples located one meter away of the trunk in the four cardinal points were collected below each tree by digging 10 × 10 × 20 cm after removing the litter layer. The four samples taken per tree were pooled into a single composite sample. Samples were kept at 4 ºC until processing, which took no longer than three weeks. Roots were separated from soil by hand, the coarse ones were discarded (diameter > 2 mm) and rhizospheric soil was detached from fine roots with the aid of forceps. Roots were then gently washed with tap water over 2 and 0.5 mm sieves for collecting fine root tips. Rhizospheric soil aliquots were collected and stored at -20 ºC for further analysis. Remaining bulk soil was air dried and sieved (2 mm) for additional physical-chemical analyses (pH and electrical conductivity, both 1:5, w:v in H_2O, organic matter and total carbon, nitrogen (Kjeldahl method), and extractable phosphorus and potassium determined by inductively coupled plasma spectrometry, Optima 4300DV, Perkin-Elmer) (Table S1). Fine roots were observed under the stereomicroscope, and a subset of ~1.5 g (fresh weight) of randomly selected ectomycorrhizal root tips per sample was frozen with liquid nitrogen, lyophilized and ground with mortar and pestle for further molecular analyses.

Molecular analyses

Genomic DNA was extracted with the DNeasy Plant Mini kit (Qiagen, Hilden, Germany) from ECM root tips (50 mg of lyophillized powder), and with the PowerSoil kit (MoBio, Carlsbad, CA, USA) from rhizospheric soil (500 mg). In both cases, to remove PCR inhibitors, ~15 mg of polyvinylpolypyrrolidone (PVPP) was added to each sample before DNA extraction. DNA extracts belonging to the three replicate trees per plant genotype and experimental block were pooled, thus making a total of 35 ECM root tip extracts and 35 rhizospheric soil extracts.

On ECM root tip DNA extracts, the internal transcribed spacer region ITS-1 of the nuclear ribosomal DNA was amplified with the primer pair ITS1F-ITS2 (Gardes and Bruns, 1993) adapted for pyrosequencing as described by Buée *et al.* (2009). PCR amplifications (3 min 94ºC,

30 cycles of 1 min 94ºC, 30 s 53ºC and 45 s 72ºC, with a final step of 10 min 72ºC) were conducted in a Verity Thermal Cycler (Life Technologies). Each sample was amplified in three independent 20 µl reactions, each containing 2 µl 10x polymerase buffer, 2.4 µl 25 mM $MgCl_2$, 1.12 µl 10 mg ml^{-1} BSA, 0.4 µl 10 mM nucleotide mix, 0.4 µl 10 mM forward and reverse primers (adaptor A-tag-ITS1F/adaptor B-ITS2), and 0.2 µl AmpliTaqGold polymerase (5 U ml^{-1}) (Applied Biosystems, Carlsbad, CA, USA). Negative controls without DNA were included in all runs to detect contaminations. Independent reactions per sample were combined, and each PCR product purified (UltraClean PCR clean-up kit, MoBio, CA, USA), quantified (PicoGreen, Life Technologies, CA, USA) and pooled in an equimolar library containing 35 uniquely tagged samples.

On rhizospheric soil DNA extracts, the 16S rRNA gene was amplified using the eubacterial primers 27F (5'- AGAGAGTTTGATCCTGGCTCAG -3') and 519R (5'- GWATTACCGCGGCKGCTG -3') (Lane, 1991) with barcodes. PCR amplifications (3 min 94ºC, 28 cycles of 30 s 94ºC, 40 s 53ºC and 1 min 72ºC, with a final step of 5 min 72ºC) were conducted using HotStarTaq Plus Master Mix Kit (Qiagen, Valencia, CA). All amplicon products from different samples were pooled in equal concentrations and purified using Agencourt Ampure beads (Agencourt Bioscience Corporation, MA, USA). Samples were sequenced using Roche 454-GS-FLX titanium instruments and reagents (Roche Applied Biosystems, USA) by the sequencing services in Parque Científico de Madrid (Spain) and MR-DNA (TX, USA).

Bioinformatic analyses and phylogeny reconstruction

A total of 106,789 and 361,880 sequences were obtained for fungal and bacterial communities, respectively. For both communities, sequences were de-multiplexed according to their tags, filtered and trimmed. For fungi, the ITS1 was extracted with the Fungal ITSx v1.0.3 (Bengtsson-Palme *et al.*, 2013). Sequences <100 bp for fungi and <350 bp for bacteria, as well as chimeric sequences and singletons were removed. Dereplication and clustering were performed with USEARCH v8.0.1616 software (Edgar, 2013). Molecular operational taxonomic units (MOTUs) were generated at 97% similarity threshold. Taxonomic assignation of representative sequences for each fungal MOTU was done by using the Basic Local Alignment Search Tool (BLAST) algorithm v2.2.23 (Altschul *et al.*, 1990) against the UNITE database (Kõljalg *et al.*, 2013). Fungal taxonomic assignment was used to identify MOTUs most closely related to known ECM taxa (Tedersoo and Smith, 2013; Tedersoo *et al.*, 2014; Nguyen *et al.*, 2016). The 75% of the sequences corresponded to ECM fungi and these were used for all subsequent analyses. For bacteria, sequences representative of each MOTU were assigned to bacterial taxa using RDP

(Wang *et al.*, 2007) at a confidence threshold of 80%. Data were deposited in the Sequence Read Archive (http://www.ncbi.nlm.nih.gov/sra) as PRJNA324224.

Fungal community phylogeny from 301 ECM fungal MOTUs was estimated with the program Phylomatic as implemented in Phylocom 4.2 (Webb *et al.*, 2008) and BEAST 1.5.4 (Drummond and Rambaut, 2007). We generated a fungal mega-tree whose topology and age estimates for major nodes were based on the phylogenetic information available in the literature (Table S2). Phylomatic takes as input a list of taxa and matches the taxa to the most resolved position in the mega-tree constructed from published phylogenies, e.g. if any genus is missing from the mega-tree, the program returns a polytomy of genera within that family (Moles *et al.*, 2005). For the remaining undated nodes, ages were estimated by using the BLADJ algorithm in Phylocom (Webb *et al.*, 2008). This program distributes undated nodes evenly between nodes of known ages. To check for the robustness of our results to the topological and chronological uncertainty introduced by the Phylomatic + BLADJ procedures, we constructed another phylogenetic tree based on a branch length adjustment procedure that follows a birth-death evolutionary model while randomly resolves the polytomies in the BEAST program (Drummond and Rambaut, 2007; Kuhn *et al.*, 2011). We ran Markov Chain Monte Carlo (MCMC) analyses for $5x10^6$ iterations, sampling trees every 10^3 iterations, discarded a 25% burnin and recovered the maximum clade credibility tree using the TreeAnnotator v1.5.4 software (Drummond and Rambaut, 2007).

The reconstruction of phylogenetic relationships in bacterial taxa was made for 2,650 bacterial MOTUs that were aligned with INFERNAL (Nawrocki and Eddy, 2013) implemented in RDP Pipeline (Cole *et al.*, 2014). The relative abundance of each MOTU was corrected by the estimated number of 16S rRNA gene copies (Kembel *et al.*, 2012). To account for uncertainty in the phylogenetic reconstruction, we built two independent maximum likelihood phylogenetic trees with the GTRGAMMA substitution model using RAxML 7.3.0 (Stamatakis, 2006), after removing the hypervariable regions with the Lane mask (Lane, 1991). Tree topology was constrained to match the basal relationships of the mega-tree of the Silva database (Quast *et al.* 2013). All phylogenetic trees were selected among the best of 10^3 iterations.

Phylogenetic community structure

We described the phylogenetic structure of fungal and bacterial communities by using the method proposed by Pillar & Duarte (2010), which has proven useful to explain ecosystem functions driven by soil microbial communities (Pérez-Valera *et al.*, 2015). This method defines the phylogenetic community structure by calculating a matrix (matrix P) that contains the composition of species fuzzy-weighed by their pairwise phylogenetic similarities (Pillar and

Duarte, 2010). We calculated matrix P by using the *PCPS* package implemented in R (Debastiani *et al.*, 2015). In matrix P, each MOTU has a value per sample that increases as the phylogenetic distance between neighbouring MOTUs decreases. Ordination techniques allow reducing matrix P to represent the phylogenetic structure at the sample level. We used Principal Coordinate Analysis (PCoA) with Euclidean distances and extracted the sample scores along the first axis, which represents the principal component phylogenetic structure (PCPS1). This axis captures the deepest phylogenetic divergences among lineages (Duarte *et al.*, 2012) and can be used in further statistical analyses as a single variable that describes the community phylogenetic structure (Pérez-Valera *et al.*, 2015). We calculated the contribution of each fungal and bacterial phylum (mean ± SE) as the loadings of each taxon to the respective PCPS1.

To check for the consistency of our results regarding the phylogenetic tree used, we performed Pearson correlations between PCPS1 calculated with both the Phylocom and BEAST methods for fungi and the two trees obtained with RAxML for bacteria. The correlation values were 0.999 for both fungi and bacteria, confirming the robustness of the analysis. For the sake of simplicity, we used a single tree for further analyses in each group.

Ecosystem functioning

The community functioning was evaluated on rhizospheric soil by measuring the activity of seven enzymes secreted by fungi and bacteria and related to C, N and P cycling, adapting the method described by Mathieu et al. (2013). Six enzymatic tests were based on fluorogenic methylumbelliferone (MU) substrate release for: β-glucosidase (EC 3.2.1.3) and cellobiohydrolase (EC 3.2.1.91) which release glucose and cellobiose respectively from cellulose; xylosidase (EC 3.2.1.37) that hydrolyses xylose from xylan; β-glucuronidase (EC 3.2.1.31) that hydrolyses β-d-glucuronic acid residues from the non-reducing terminal of glycosaminoglycan; acid phosphatase (EC 3.1.3.2) involved in the breakdown of phosphoric ester bonds and release of phosphate ions; chitinase (EC 3.2.1.14) which hydrolyses glycosidic bonds in chitin. A seventh enzymatic test was based on fluorogenic methylcoumarine (AMC) substrate release for L-leucine aminopeptidase (EC 3.4.11.1) that can remove the N-terminal l-leucine from peptidic substrates. Measurements were done in a Victor microplate reader (Perkin-Elmer Life Sciences, Massachusetts, USA), with 355/460 nm excitation/emission wavelengths.

Statistical analyses

We first tested the existence of spatial autocorrelation on the phylogenetic community structure of microbial communities through mantel tests using the vegan package for R 3.1.1 (R Core Team, 2014; Oksanen *et al.*, 2015). We did not find spatial autocorrelation either for ECM

fungi or bacteria (mantel correlations for all sites yielding $p>0.05$), and therefore we did not include the geographical coordinates of the trees in further statistical analyses.

In order to determine whether the plant genotype had an effect on the phylogenetic structure of root-associated microbial communities, we ran Bayesian generalized linear models (GLM) using the MCMCglmm package for R (Hadfield, 2010). We used fungal PCPS1 or bacterial PCPS1 as dependent variables in two separate models, and plant genotype and planting site as fixed factors. We used the default priors and ran 13,000 MCMC iterations with a burn-in period of 3,000 iterations. The statistical significance of the factors in the model was estimated by calculating the 95% credible interval of their posterior distribution. To test for the existence of a significant site × genotype interaction we used the Deviance Information Criterion (DIC) for comparison of the models with and without the interaction. The site × genotype interaction was not significant in any case (data not shown) and was not further considered.

We also tested whether the phylogenetic structure of microbial communities predicted the ecosystem functioning with Bayesian GLMs as above. To do so, we ran MCMCglmms using each ecosystem function, i.e. enzymatic activities related to C, N and P cycles taken individually as the dependent variable, and fungal PCPS1, bacterial PCPS1 and planting site as fixed factors in the same model. Interactions between site and each PCPS1 were tested as above and found to be non-significant (data not shown). Finally, to determine whether ECM fungi and rhizospheric bacteria show parallel phylogenetic community structures, we correlated the fungal and bacterial PCPS1.

To ensure that tree genotype differences on either the phylogenetic structure of microbial communities or ecosystem functions were not a consequence of the environmental similitude between the tree geographic origin and the planting sites, we compared the fungal and bacterial PCPS1 as well as the enzymatic activities of each genotype with the environmental distances between the planting site and geographic origin (see Hernández-Serrano *et al.* 2014 for a similar procedure). We used elevation (m), mean annual precipitation (mm), mean annual temperature (ºC), mean of daily maximum of the month of highest average (ºC) and mean of daily minimum of month of lowest average (ºC) to calculate environmental distances with the help of Gower index, which has good performance in detecting underlying ecological gradients (Faith *et al.*, 1987). Climatic data were taken from Alía *et al.* (1997). None of the correlations were significant ($p>0.05$), indicating that neither the phylogenetic structure of microbial communities nor the enzymatic activities depends on the environmental similarity between the geographic origin and the planting sites.

RESULTS

A total of 75,872 and 133,581 final sequences were obtained after post-processing for ECM fungal and bacterial rhizospheric communities, respectively. Sequence grouping yielded a total of 301 ECM fungal (60±11 per sample) and 2,650 bacterial (501±14 per sample) MOTUs. ECM fungal MOTUs were assigned to the phyla Ascomycota (11%) or Basidiomycota (89%) (Table S3). We identified 14 bacterial phyla, including Proteobacteria (33%), Actinobacteria (18.8%), Planctomycetes (15.4%), Acidobacteria (13%) and Bacteroidetes (5.2%) (Table S4).

Microbial phylogenetic community structure

The analysis of matrix P values showed a differential contribution of ECM fungal and bacterial phyla, respectively, to the overall phylogenetic community structure (Figure S1). Ascomycota showed high matrix P scores indicating a tendency of these fungi to co-exist with close relatives compared to Basidiomycota. For Bacteria, MOTUs assigned to Proteobacteria, Firmicutes, Acidobacteria and the candidate phylum Saccharibacteria showed the highest matrix P values on average, while those within Bacteroidetes and Planctomycetes had the lowest scores.

The first principal component of the phylogenetic community structure (PCPS1) that captures deep phylogenetic differences among lineages, explained 50% and 41% of the total variance of ECM fungal and bacterial communities, respectively. The contribution of fungal phyla to PCPS1 revealed a preponderance of Basidiomycota on the negative pole of the axis, while Ascomycota were positioned on the positive pole (Figure 2a). For bacteria, a clear segregation was observed associating negative PCPS1 with Proteobacteria and positive PCPS1 with the other phyla (Figure 2b).

Plant genotype effects on microbial phylogenetic community structure

The plant genotype, along with the planting site, determined the microbial phylogenetic community structure as follows. The planting site significantly explained the phylogenetic structure of both ECM fungal and bacterial communities (Figure 2; Table S5), and this effect was similar across plant genotypes as revealed by the non-significant site × genotype interaction. Most interestingly, the plant genotype significantly explained the phylogenetic structure of ECM fungal and bacterial communities (Figure 2; Table S5). Specifically, we detected divergent fungal phylogenetic assemblages under Mediterranean trees (i.e. overrepresentation of Basidiomycetes, as indicated by the negative scores on PCPS1) compared with the Atlantic genotype (i.e. overrepresentation of Ascomycetes) (Figure 2a). The fungal assemblages of African trees did not significantly differ from either of the other plant genotypes (Figure 2a). The phylogenetic structure of bacterial communities under the Mediterranean genotype was significantly different from those of the Atlantic and African genotypes, the former showing negative PCPS1 values that

indicate an overrepresentation of Proteobacteria (Figure 2b). Additionally, the phylogenetic structure of the ECM fungal and bacterial communities was significantly correlated (r=0.55, P<0.001).

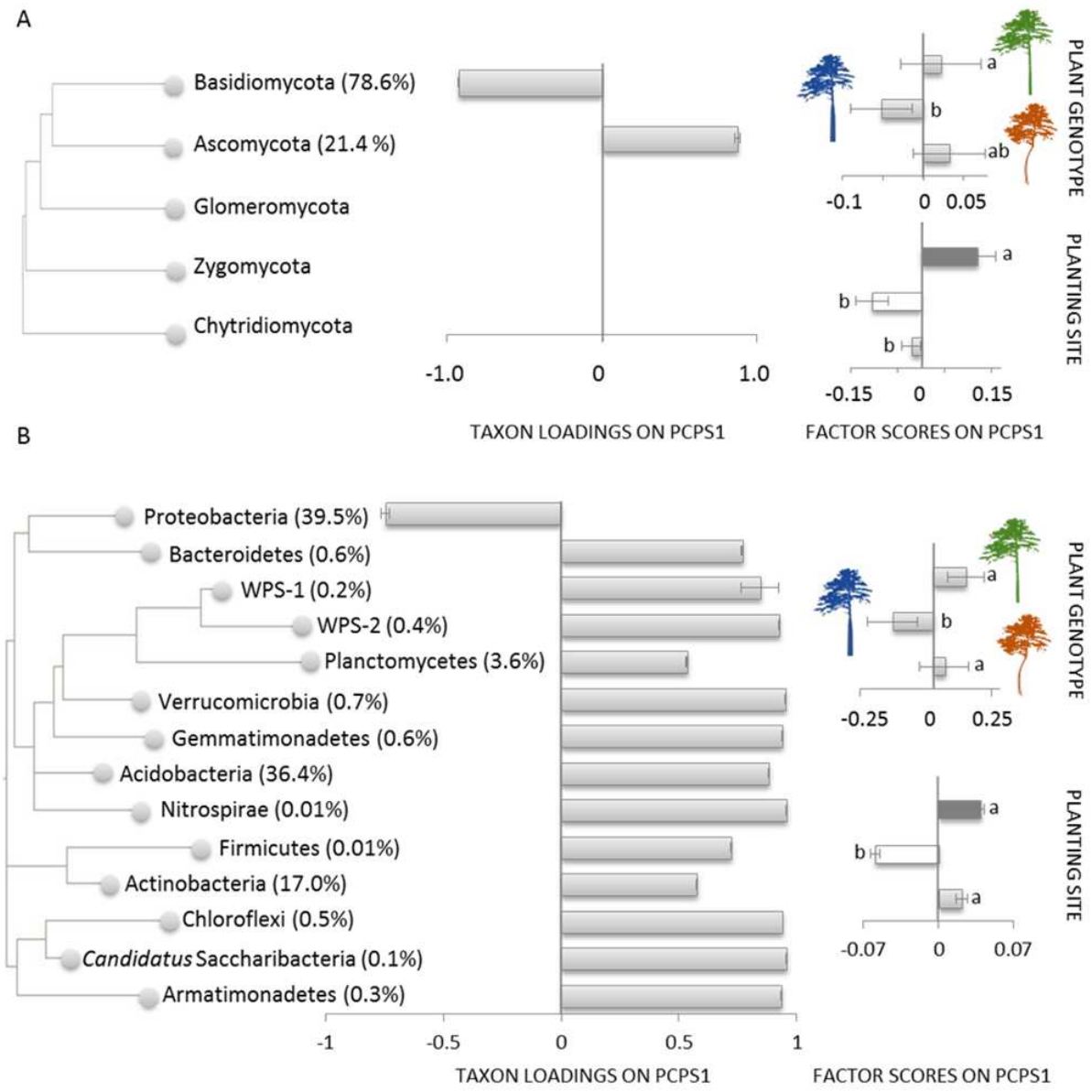

Figure 2 | Effect of the plant genotype and planting site on the phylogenetic structure of (a) ECM fungal and (b) bacterial communities. From left to right, i) Phylogenetic trees depicting the relationships between main phyla (relative abundance in parentheses); ii) Loadings (means ± SE) of each taxon on PCPS1; iii) Scores of plant genotype and planting site on PCPS1. Pine silhouettes depict different *P. pinaster* genotypes (as in Figure 1). Colours indicate different sites (dark grey: Cabañeros, white: Riofrío; light grey: Espinoso del Rey). Different letters denote significant differences among genotypes or sites according to Bayesian GLMs (see Table S5).

Table 1 | Bayesian post-mean estimates and their 95% expected credible intervals (in brackets) of the effect of ECM fungal and bacterial phylogenetic community structure (PCPS1) and planting site on enzymatic activities. The site Cabañeros was taken as the reference in all models. Significant differences (i.e. credible intervals not including zero) are shown in bold type.

Nutrient Cycle	Enzymatic activity	Fungal PCPS1	Bacterial PCPS1	Site Espinoso	Site Riofrío
C (cellulose)	**Glucosidase**	-0.25 [-0.71, 0.19]	-1.71 [-3.73, 0.26]	-0.04 [-0.18, 0.11]	-0.18 [-0.44, 0.04]
	Cellobiohydrolase	**-0.06 [-0.12,-0.003]**	-0.21 [-0.48, 0.08]	-0.003 [-0.02, 0.02]	-0.03 [-0.06, 0.005]
C (hemicellulose)	**Xylosidase**	-0.003 [-0.03,0.021]	-0.004 [-0.13,0.13]	0.004 [-0.004,0.013]	-0.003 [-0.02, 0.01]
	Glucuronidase	0.43 [-0.18, 1.02]	**-2.39 [-5.15, -0.04]**	-0.12 [-0.28, 0.08]	-0.18 [-0.50, 0.12]
N (peptides)	**Aminopeptidase**	**0.028 [0.005, 0.05]**	0.012 [-0.09, 0.10]	0.006 [-0.001, 0.01]	0.002 [-0.01, 0.01]
N (chitin)	**Chitinase**	0.034 [-0.22, 0.30]	**-1.08 [-2.24, -0.01]**	**-0.17 [-0.25, -0.08]**	**-0.23 [-0.36, -0.09]**
P	**Phosphatase**	-0.33 [-2.38, 1.47]	-1.20 [-10.0, 7.58]	**-2.02 [-2.60, -1.29]**	**-1.57 [-2.66, -0.53]**

Microbial phylogenetic community structure effects on ecosystem functioning

The enzymatic activities measured in the rhizospheric soils showed great variability across plant genotypes (Figure 3). In order to explain this variability, we tested the effects of the phylogenetic structure of ECM fungal and bacterial communities, which significantly predicted the activity of several enzymes that mediate the C and N cycles (Table 1). P cycling, however, was only explained by the planting site (Table 1). Fungal PCPS1 exerted a significantly negative effect on cellobiohydrolase activity, indicating that samples with high relative abundance of Basidiomycetes also showed faster rates of cellulose degradation (Table 1; Figure 2a). On the contrary, fungal PCPS1 had a significantly positive effect on leucine activity mediated by the overrepresentation of Ascomycetes (Table 1; Figure 2a). On the other side, the bacterial phylogenetic structure explained the activity of glucuronidase and chitinase (Table 1). The relationship between both enzymes and the bacterial PCPS1 was negative suggesting an important contribution of Proteobacteria to their activity. Additionally, the planting site had a significant effect on chitinase (Table 1).

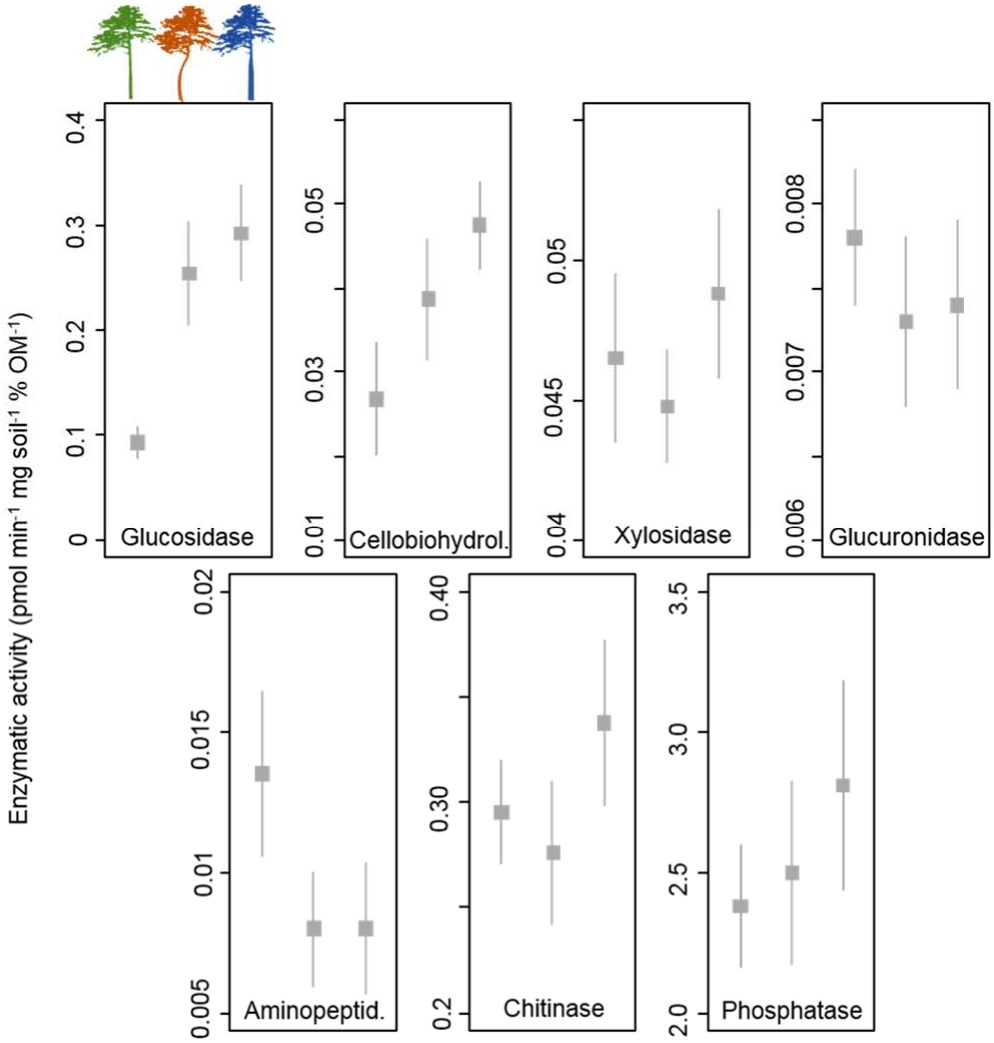

Figure 3 | Enzymatic activities related to C, N and P cycling in the rhizosphere of three *P. pinaster* genotypes (as in Figure1). Bars indicate standard errors. See Table 1 for plant genotype and planting site statistical effects.

DISCUSSION

Our long-term common garden experiments indicate that, together with a site effect, the genotype of *P. pinaster* trees significantly explains the phylogenetic structure of root-associated ectomycorrhizal and bacterial communities. The phylogenetic structure of these microbial communities has further consequences on ecosystem performance related to nutrient cycling. In particular, ECM fungal and bacterial communities significantly predicted enzymatic activities relevant to the C and N cycles, while variations in P cycling were exclusively related to the planting sites. Interestingly, fungi and bacteria explained the activity of different C and N enzymes, suggesting functional complementarity of both groups. Our study provides valuable insights about plant-microbiota interactions under well replicated field conditions, thus overriding the limitations of studies that are performed under laboratory conditions or that focus on a single group of microbes, as discussed by van der Putten *et al.* (2013) and van der Heijden *et al.* (2015).

The local environmental context has been recognized as a main factor determining the effect of plant species identity or genotype on the microbial community structure (Peiffer *et al.*, 2013; Tedersoo *et al.*, 2016). Our results also indicate that local processes exert a strong effect in phylogenetically structuring the ECM fungal and bacterial communities thriving in the rhizosphere, as the planting site was a significant source of variation in our experiment. Despite this site effect, we detected that the plant genotype effect on the microbial community was consistent across planting sites.

The genotype of *P. pinaster* significantly determined the phylogenetic community structure of the ECM fungal and bacterial communities in its rhizosphere, regardless of the environmental conditions. The Atlantic and Mediterranean genotypes sheltered the most distinct microbial communities. The ECM fungal phylogenetic assemblage of Mediterranean trees was dominated by Basidiomycetes, which tended to co-exist with evolutionarily distant fungi, compared to that of Atlantic trees, which showed an overrepresentation of Ascomycetes that predominantly co-occurred with closer relatives. Mycorrhizal symbioses are generally seen as non-specific interactions, a generalist fungal and host strategy that enhances plant success into diverse habitats (Kennedy *et al.*, 2003; Tedersoo *et al.*, 2014). Multiple ECM fungi can interact with the same tree at the root interface aided by a broad compatibility recognition and priority effects (Molina *et al.*, 1992; Kennedy *et al.*, 2009). Despite the generally low specificity in mycorrhizal symbioses, the carbon allocated to each fungi for mycelial biomass can greatly differ depending on their exploration strategy (Agerer, 2001; Courty *et al.*, 2016), nutrient mobilization ability (Plassard *et al.*, 2011; Talbot *et al.*, 2015) and on whether they are favoured by the host (Cullings *et al.*, 2001; Bever *et al.*, 2009). Even environmental stressors, often associated with a reduced plant photosynthetic activity, can alter the ECM fungal communities particularly by

favouring Ascomycetes (Brown *et al.*, 2001; Mueller and Gehring, 2006; Rincón *et al.*, 2014). In line with these studies, we interpret that the plant genotype, by determining resource allocation to its symbionts, may influence the phylogenetic community structure of ECM fungi. This phylogenetic structure, in turn, allows predicting enzyme activities targeting carbohydrates (cellulose) and small peptides. In root tips, ECM fungal cellulolytic activities are needed to disrupt plant cell walls (Pritsch and Garbaye, 2011), and the use of proteins as the unique nitrogen source by ECM fungi has often been reported (Read and Perez-Moreno, 2003; Talbot and Treseder, 2010). Here, we could attribute the increased cellulolytic activity to the overrepresentation of Basidiomycetes underneath the productive Mediterranean genotype, and the peptidase activity to the dominance of Ascomycetes in the roots of the Atlantic genotype. These enzymes are relevant not only to nutrient cycling but also to the tree-fungus symbiosis, as trees can invest more than 30 % of the carbon fixed to maintain their associated ECM fungi, which reciprocate with the uptake of limiting nutrients (Smith and Read, 2008). Tree productivity is frequently N-limited and the role of ECM fungi in mobilizing N for their host is fundamental (Read and Perez-Moreno, 2003; Clemmensen *et al.*, 2013; Averill *et al.*, 2014). Thus, the activity of amino peptidases, particularly those of Ascomycetes, in the rhizosphere could be of main importance both for the tree, to increase the uptake of labile forms of nitrogen as amino acids, and for the fungus to maintain the C flux (Tedersoo and Smith, 2013; Lamit *et al.*, 2016).

The tree host genotype not only affected the fungal community that interacted directly with its roots, but also determined the phylogenetic assembly of the bacterial community proliferating in the rhizosphere. This observation can be most likely explained by the fact that the concentration, composition and quality of rhizodeposits determine the abundance and diversity of bacteria in the rhizosphere (Bulgarelli *et al.*, 2013; Edwards *et al.*, 2015; Steinauer *et al.*, 2016). In particular, the effect of root exudates on the structure and activity of rhizospheric bacteria has been attributed to the variation in genes responsible for the plant C allocation strategy (Aira *et al.* 2010). In this study, we found that bacterial communities associated with the Mediterranean genotype of *P. pinaster*, which was the most productive in terms of biomass, had an overrepresentation of Proteobacteria that showed low phylogenetic distances to their neighbours. Proteobacteria includes fast-growing microbes that feed on carbon sources of varying recalcitrance and outcompete distantly-related bacterial lineages (Fierer *et al.*, 2007; Goldfarb *et al.*, 2011). This ability leads them to dominate carbon-rich soil environments where they tend to co-exist with close relatives (Goberna, García, *et al.*, 2014; Goberna, Navarro-Cano, *et al.*, 2014). Indeed, different studies revealed a significant enrichment of Proteobacteria in the carbon-rich rhizosphere compared to the surrounding bulk soils (Uroz *et al.*, 2016). Thus, our results suggest that the productive Mediterranean genotype would be able to produce more photoassimilates

and/or to redirect more in form of root exudates (Farrar *et al.*, 2003) promoting the proliferation of these competitive bacterial clades. The overrepresentation of Proteobacteria was further related to an increased enzymatic cleavage of branched carbohydrates (hemicellulose) and recalcitrant N compounds (chitin). Interestingly, Uroz *et al.* (2013) reported that the ectomycorrhizosphere of forest trees appeared significantly enriched in Proteobacterial isolates capable of hydrolysing chitin. The production of chitinases among bacteria is ecologically relevant since they are involved in N mobilisation primarily by targeting chitin but also by decomposing peptidoglycan, which respectively constitute abundant components of fungal and bacterial cell walls (Islam and Datta, 2015). This enzymatic activity could therefore underlie not only N cycling but also ecological interactions, particularly pathogenicity, both among bacteria and between bacteria and fungi (Frederiksen *et al.*, 2013).

Ecological interactions between fungi and bacteria are of key importance in the rhizospheric environment (van der Heijden and Schlaeppi, 2015). Indeed, we found a highly significant correlation between the phylogenetic community structure of symbiotic ECM fungi and rhizospheric bacteria. This observation fits well with the reported influence of ECM fungi in structuring their associated bacteria in the mycorrhizosphere (Frey-Klett *et al.*, 2007; Deveau, 2016), and with the idea that the rhizobacterial habitat can be highly specific at the microsite scale (Edwards *et al.*, 2015). Our results, therefore, add on this evidence and further show that the plant genotype can be a main driver of the assembly of the fungal and bacterial communities interacting in the rhizosphere. It is obvious that plants have evolved complex interactions with their root-associated microbiota, which can in turn remarkably modify the plant phenotype (Friesen, 2013). Similarly to humans that emit their own microbial cloud (Meadow *et al.*, 2015), plants also maintain a rhizospheric microbial cloud through which they can modulate key ecosystem processes that ultimately determine their survival and adaptation to the environment (Vandenkoornhuyse *et al.*, 2015).

SUPPORTING INFORMATION

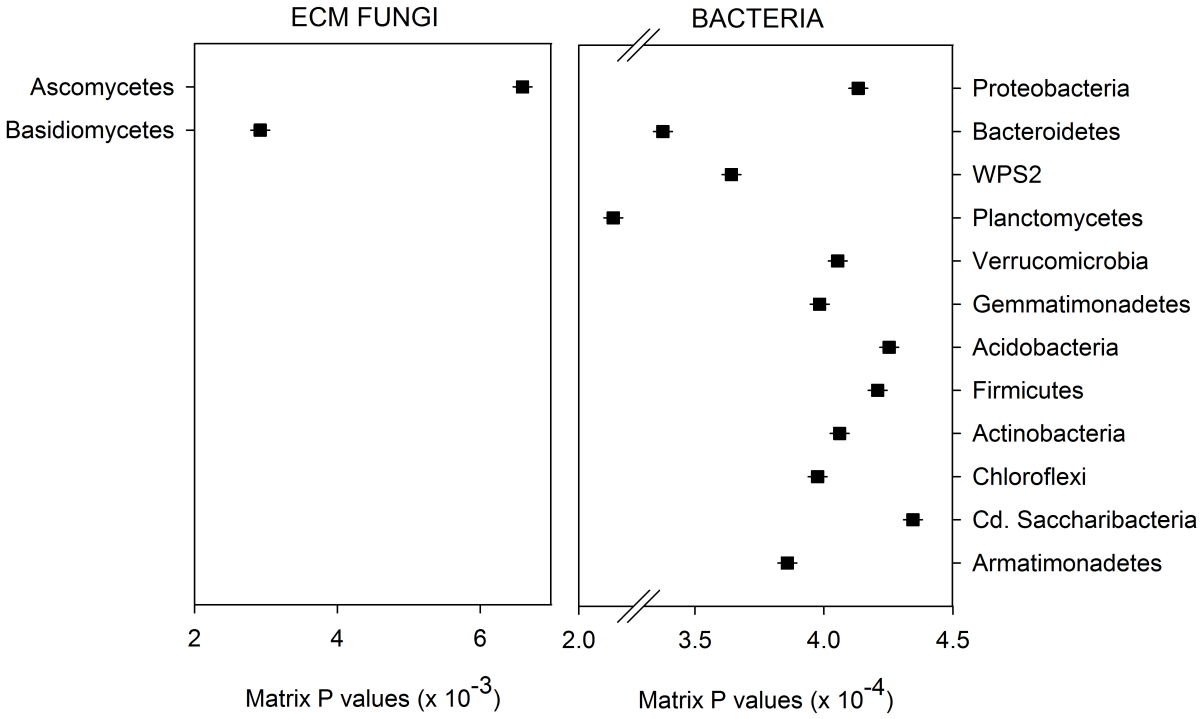

Figure S1 | Scores of main ECM fungal and bacterial phyla in matrix P (see main text for a detailed explanation). Values are means ± SD.

Table S1 | Soil properties (means ± SE, n =35) and climatic features in each study site. EC = electric conductivity, OM = organic matter, N = nitrogen, P = phosphorous, K = potassium, C:N = carbon/nitrogen ratio. Data in Table S1 (Chapter 1) Climatic data extracted from Alía *et al* 1997.

	pH	EC (µS/cm)	OM (%)	N (%)	P (mg/kg)	K (mg/kg)	C:N	Altitude (m)	Precipitation (mm)	Mean Temperature (°C)
Cabañeros	4.9 ± 0.1	77.2 ± 5.7	8.8 ± 0.4	0.20 ± 0.01	5.4 ± 0.2	31.3 ± 1.7	26.2 ± 0.9	1045	800	12.8
Riofrío	5.3 ± 0.1	72.2 ± 2.9	7.9 ± 0.4	0.17 ± 0.01	4.3 ± 0.2	55.4 ± 3.5	27.8 ± 0.7	775	724	10.2
Espinoso del Rey	5.1 ± 0.1	72.5 ± 3.2	7.5 ± 0.5	0.15 ± 0.01	4.7 ± 0.2	112.8 ± 5.9	28.6 ± 0.6	830	716	13.4

Table S2 | Bibliographic references used to infer the relationships among distantly related taxa within the Fungal Kingdom and the age for major nodes in the phylogenetic "megatree".

Kingdom	Phylum	Subphylum	Class/Subclass	Order	Family	
Fungi						
Ebersberger *et al.* (2012) Hibbet *et al.* (2007) James *et al.* (2006) Larsson *et al.* (2007) Tedersoo *et al.* (2010) Tehler *et al.* (2003)	**Basidiomycota** Matheny *et al.* (2007)	**Agaricomycotina** Hibett (2006) Hibbet *et al.* (2014)	**Homobasiodiomycetes** Binder *et al.* (2005) Bodensteiner *et al.* (2004) Larsson *et al.* (2004)			
			Agaricomycetes Floudas *et al.* (2012) Hibbet *et al.* (2014)	**Agaricales** Mattheny *et al.* (2006)	Agaricaceae Entolomataceae Hygrophoraceae Inocybaceae Tricholomataceae	Vellinga (2004; 2011) Baroni and Matheny (2011) Lodge *et al.* (2014) Alvarado *et al.* (2010) Sanchez-García *et al.* (2014)
				Atheliales Kotiranta *et al.* (2011) Larsson *et al.* (2004)		
				Boletales Binder and Hibett (2006) Wilson *et al.* (2012)	Boletaceae	Wu *et al.* (2014)
				Cantharellales Diederich *et al.* (2014) Moncalvo *et al.* (2006)		
				Russulales Miller *et al.* (2006)		
				Sebacinales Oberwinkler *et al.* (2014) Selosse *et al.* (2009)		
				Thelephorales Larsson *et al.* (2004)	Thelephoraceae	Tedersoo *et al.* (2014)
	Ascomycota Schoch *et al.* (2009)		**Pezizomycetes** Perry *et al.* (2007)		Pyronemataceae	Hansen *et al.* (2013); Sbissi *et al.* (2010)
Node age datation						
Amo de Paz *et al.* (2011) Beimforde *et al.* (2014) Berbee and Taylor (2010) Chen *et al.* (2015) Floudas (2012) Hedges (2015) Kohler *et al.* (2015) Rouxel *et al.* (2011)						

Table S3 | Ectomycorrhizal fungal MOTUs assigned to phylum, order, family and genus.

PHYLUM (2)	MOTUs Total
Ascomycota	33
Basidiomycota	268
Total MOTUs	301

PHYLUM	% Total
Ascomycota	11.0
Basidiomycota	89.0
Total MOTUs	100.0

ORDER (10)	MOTUs Total
Agaricales	76
Atheliales	42
Boletales	14
Cantharellales	9
Helotiales	6
Hysteriales	7
Pezizales	15
Russulales	35
Sebacinales	20
Thelephorales	72

FAMILY (26)	MOTUs Total
Amanitaceae	5
Atheliaceae	42
Bankeraceae	9
Boletaceae	2
Cantharellaceae	1
Clavulinaceae	7
Cortinariaceae	14
Entolomataceae	3
Gloniaceae	7
Gomphidiaceae	1
Helotiaceae	3
Hydnaceae	1
Hydnangiaceae	3
Hygrophoraceae	1
Inocybaceae	41
Pezizaceae	3
Pyronemataceae	5
Rhizopogonaceae	8
Russulaceae	35
Sebacinaceae	19
Strophariaceae	3
Suillaceae	3
Thelephoraceae	63
Tricholomataceae	6
Tuberaceae	7
Vibrisseaceae	1

GENUS (39)	MOTUs Total
Amanita	5
Amphinema	13
Cadophora	1
Cantharellus	1
Cenococcum	7
Chroogomphus	1
Clavulina	7
Cortinarius	11
Entoloma	3
Geopora	1
Hebeloma	3
Hydnellum	8
Hydnum	1
Hygrophorus	1
Hymenoscyphus	2
Inocybe	37
Laccaria	3
Lactarius	9
Meliniomyces	5
Phaeangium	3
Phellodon	1
Phialocephala	1
Piloderma	4
Pseudotomentella	5
Rhizopogon	8
Rhizoscyphus	1
Rozites	1
Russula	19
Sebacina	7
Suillus	3
Terfezia	3
Thelephora	3
Tomentella	22
Tomentellopsis	4
Tricholoma	6
Tuber	7
Tylospora	25
Wilcoxina	1
Xerocomus	2

Table S4 | Bacterial MOTUs assigned to phylum, class, order, family and genus.

PHYLUM (14)	MOTUs
Proteobacteria	874
Actinobacteria	497
Planctomycetes	407
Acidobacteria	345
Bacteroidetes	139
Verrucomicrobia	89
Armatimonadetes	88
Chloroflexi	75
Candidatus Saccharibacteria	37
Candidate division WPS-2	35
Gemmatimonadetes	27
Candidate division WPS-1	26
Firmicutes	10
Nitrospirae	1
Total MOTUs	2650

PHYLUM	%
Proteobacteria	33.0
Actinobacteria	18.8
Planctomycetes	15.4
Acidobacteria	13.0
Bacteroidetes	5.2
Verrucomicrobia	3.4
Armatimonadetes	3.3
Chloroflexi	2.8
Candidatus Saccharibacteria	1.4
Candidate division WPS-2	1.3
Gemmatimonadetes	1.0
Candidate division WPS-1	1.0
Firmicutes	0.4
Nitrospirae	0.0
Total MOTUs	100

CLASS (42)	MOTUs
Alphaproteobacteria	489
Actinobacteria	468
Planctomycetia	404
Deltaproteobacteria	112
Acidobacteria_Gp1	108
Sphingobacteriia	89
Gammaproteobacteria	79
Betaproteobacteria	74
Acidobacteria_Gp3	72
Ktedonobacteria	68
Acidobacteria_Gp2	44
Saccharibacteria_genera_incertae_sedis	37
WPS-2_genera_incertae_sedis	35
Armatimonadia	32
Acidobacteria_Gp6	30
Acidobacteria_Gp4	29
Spartobacteria	29
Gemmatimonadetes	27
Opitutae	26
WPS-1_genera_incertae_sedis	26
Chthonomonadetes	24
Armatimonadetes_gp5	18
Acidobacteria_Gp16	17
Subdivision3	17
Acidobacteria_Gp7	11
Acidobacteria_Gp5	10
Acidobacteria_Gp10	9
Bacilli	8
Acidobacteria_Gp15	7
Bacteroidetes_incertae_sedis	5
Armatimonadetes_gp4	3
Flavobacteriia	3
Phycisphaerae	3
Verrucomicrobiae	3
Acidobacteria_Gp17	2
Thermomicrobia	2
Caldilineae	1
Chloroflexia	1
Clostridia	1
Cytophagia	1
Fimbriimonadia	1
Nitrospira	1
Unidentified	224

ORDER (64)	MOTUs
Planctomycetales	404
Actinomycetales	265
Rhizobiales	145
Rhodospirillales	115
Sphingobacteriales	89
Solirubrobacterales	88
Myxococcales	85
Gp3	65
Gp1	63
Ktedonobacterales	63
Gaiellales	48
Gp2	44
Acidimicrobiales	40
Saccharibacteria_genera_incertae_sedis	37
WPS-2_genera_incertae_sedis	35
Armatimonadales	32
Burkholderiales	30
Gp6	30
Spartobacteria_genera_incertae_sedis	29
Gp4	28
Gemmatimonadales	27
WPS-1_genera_incertae_sedis	26
Opitutales	25
Chthonomonadales	24
Xanthomonadales	22
Caulobacterales	21
Legionellales	20
Armatimonadetes_gp5	18
Gp16	17
Subdivision3_genera_incertae_sedis	17
Alphaproteobacteria_incertae_sedis	13
Sphingomonadales	12
Gp7	11
Gp5	10
Granulicella	10
Gp10	9
Bacillales	7
Gp15	7
Ohtaekwangia	5
Armatimonadetes_gp4	3
Candidatus Solibacter	3
Flavobacteriales	3
Phycisphaerales	3
Verrucomicrobiales	3
Bdellovibrionales	2
Enterobacteriales	2
Gp17	2
Neisseriales	2
Pseudomonadales	2
Acidicapsa	1
Blastocatella	1
Caldilineales	1
Candidatus Koribacter	1
Chloroflexales	1
Clostridiales	1
Cytophagales	1
Fimbriimonadales	1
Nitrospirales	1
Procabacteriales	1
Rhodobacterales	1
Rhodocyclales	1
Rickettsiales	1
Telmatobacter	1
Terriglobus	1
Unidentified	574

Continuation **Table S4**

FAMILY (100)	MOTUs
Planctomycetaceae	404
Acetobacteraceae	80
Chitinophagaceae	74
Gp3	65
Gp1	63
Gaiellaceae	48
Gp2	44
Saccharibacteria_genera_incertae_sedis	37
WPS-2_genera_incertae_sedis	35
Armatimonadaceae	32
Gp6	30
Spartobacteria_genera_incertae_sedis	29
Gp4	28
Polyangiaceae	28
Gemmatimonadaceae	27
WPS-1_genera_incertae_sedis	26
Opitutaceae	25
Pseudonocardiaceae	25
Chthonomonadaceae	24
Mycobacteriaceae	21
Caulobacteraceae	20
Nocardioidaceae	20
Armatimonadetes_gp5	18
Gp16	17
Streptomycetaceae	17
Subdivision3_genera_incertae_sedis	17
Xanthomonadaceae	17
Rhodospirillaceae	16
Sphingobacteriaceae	15
Bradyrhizobiaceae	13
Coxiellaceae	12
Rhizomicrobium	12
Solirubrobacteraceae	12
Sphingomonadaceae	12
Cystobacteraceae	11
Gp7	11
Beijerinckiaceae	10
Gp5	10
Granulicella	10
Thermosporotrichaceae	10
Conexibacteraceae	9
Gp10	9
Acidimicrobineae_incertae_sedis	8
Legionellaceae	8
Micromonosporaceae	8
Gp15	7
Oxalobacteraceae	7
Actinospicaceae	6
Burkholderiaceae	6
Microbacteriaceae	6
Comamonadaceae	5
Iamiaceae	5
Ktedonobacteraceae	5
Ohtaekwangia	5
Sinobacteraceae	5
Xanthobacteraceae	5
Hyphomicrobiaceae	4
Rhizobiaceae	4
Armatimonadetes_gp4	3
Candidatus Solibacter	3
Catenulisporaceae	3
Flavobacteriaceae	3
Kineosporiaceae	3
Patulibacteraceae	3
Phycisphaeraceae	3

GENUS (133)	MOTUs
Gp3	65
Gp1	63
Gaiella	48
Gp2	44
Saccharibacteria_genera_incertae_sedis	37
WPS-2_genera_incertae_sedis	35
Armatimonas/Armatimonadetes_gp1	32
Gp6	30
Spartobacteria_genera_incertae_sedis	29
Gp4	28
Gemmatimonas	27
WPS-1_genera_incertae_sedis	26
Chthonomonas/Armatimonadetes_gp3	24
Opitutus	24
Gemmata	23
Mycobacterium	21
Armatimonadetes_gp5	18
Gp16	17
Subdivision3_genera_incertae_sedis	17
Mucilaginibacter	15
Aquisphaera	13
Telmatocola	13
Aquicella	12
Rhizomicrobium	12
Solirubrobacter	12
Gp7	11
Gp5	10
Granulicella	10
Thermosporothrix	10
Caulobacter	9
Conexibacter	9
Gp10	9
Aciditerrimonas	8
Legionella	8
Gp15	7
Singulisphaera	7
Streptomyces	7
Terrimonas	7
Actinospica	6
Burkholderia	5
Iamia	5
Ktedonobacter	5
Ohtaekwangia	5
Sphingomonas	5
Zavarzinella	5
Ferruginibacter	4
Labrys	4
Marmoricola	4
Nocardioides	4
Pseudonocardia	4
Rhizobium	4
Acidocella	3
Amycolatopsis	3
Armatimonadetes_gp4	3
Bradyrhizobium	3
Candidatus Solibacter	3
Catenulispora	3
Dongia	3
Flavobacterium	3
Hydrocarboniphaga	3
Kineosporia	3
Kribbella	3
Patulibacter	3
Phenylobacterium	3
Phycisphaera	3

Continuation **Table S4**

FAMILY (100)	MOTUs
Propionibacteriaceae	3
Verrucomicrobiaceae	3
Bdellovibrionaceae	2
Burkholderiales_incertae_sedis	2
Enterobacteriaceae	2
Geodermatophilaceae	2
Gp17	2
Neisseriaceae	2
Nocardiaceae	2
Paenibacillaceae 1	2
Phyllobacteriaceae	2
Pseudomonadaceae	2
Streptosporangiaceae	2
Acidicapsa	1
Bacillaceae 1	1
Blastocatella	1
Caldilineaceae	1
Candidatus Koribacter	1
Cellulomonadaceae	1
Chloroflexaceae	1
Fimbriimonadaceae	1
Methylobacteriaceae	1
Methylocystaceae	1
Nakamurellaceae	1
Nitrospiraceae	1
Phaselicystidaceae	1
Planococcaceae	1
Procabacteriaceae	1
Rhodobacteraceae	1
Rhodobiaceae	1
Rhodocyclaceae	1
Rickettsiaceae	1
Telmatobacter	1
Terriglobus	1
Thermomonosporaceae	1
Unidentified	1042

GENUS (133)	MOTUs
Actinomycetospora	2
Flavisolibacter	2
Friedmanniella	2
Gp17	2
Massilia	2
Pelomonas	2
Pirellula	2
Pseudomonas	2
Rhodopila	2
Rhodoplanes	2
Rudaea	2
Schlesneria	2
Sorangium	2
Streptacidiphilus	2
Vampirovibrio	2
Acidicapsa	1
Acidisoma	1
Actinoallomurus	1
Asticcacaulis	1
Bacillus	1
Blastocatella	1
Blastococcus	1
Buttiauxella	1
Candidatus Koribacter	1
Candidatus Procabacter	1
Catellatospora	1
Chitinophaga	1
Collimonas	1
Dactylosporangium	1
Dokdonella	1
Duganella	1
Endobacter	1
Fimbriimonas	1
Flavitalea	1
Georgfuchsia	1
Hyalangium	1
Hyphomicrobium	1
Ideonella	1
Inquilinus	1
Isosphaera	1
Kitasatospora	1
Kutzneria	1
Leifsonia	1
Luteibacter	1
Mesorhizobium	1
Methylobacterium	1
Methylocapsa	1
Methylocystis	1
Nakamurella	1
Nevskia	1
Niastella	1
Nitrobacter	1
Nitrospira	1
Oerskovia	1
Parvibaculum	1
Pedomicrobium	1
Phaselicystis	1
Polaromonas	1
Propionibacterium	1
Pseudolabrys	1
Rhodococcus	1
Sediminibacterium	1
Segetibacter	1
Sphingobium	1
Streptosporangium	1
Telmatobacter	1
Terriglobus	1
Variovorax	1
Unidentified	1668

Table S5 | Bayesian post-mean estimates and their 95% expected credible intervals (in brackets) of the effect of plant genotype and the planting site on the ECM fungal and bacterial phylogenetic community structure (PCPS1). The Mediterranean genotype and the site Cabañeros were taken as the reference in all models. Significant differences (i.e. credible intervals not including zero) are shown in bold type.

	Genotype Atlantic	**Genotype African**	**Site Espinoso**	**Site Riofrío**
Fungal PCPS1	**0.08 [1.3×10^{-4}, 0.19]**	0.07 [-0.03, 0.17]	**-0.13 [-0.24, -0.04]**	**-0.22 [-0.31, -0.12]**
Bacterial PCPS1	**0.03 [4.9×10^{-3}, 0.05]**	**0.02 [3.3×10^{-3}, 0.04]**	-0.02 [-0.04, 5×10^{-3}]	**-0.10 [-0.12, -0.08]**

Chapter 4

Plant-soil feedbacks regulate nutrient cycling through the phylogenetic adjustment of ectomycorrhizal and saprotrophic fungal guilds

INTRODUCTION

Fungi are fundamental actors in forests involved in the carbon turnover and the mobilization of nutrients (Smith and Read, 2008). They differentially interact with trees depending on their life-style, therefore playing different ecological roles (e.g. mycorrhizal, saprotrophic, pathogenic, lichenic, endophytic) (Tedersoo and Smith, 2013). The ectomycorrhizal (ECM) fungi are particularly influential on the host fitness because they improve the uptake of water and nutrients via the external mycelium, which greatly extends the ability of roots to explore the surrounding soil. In turn, the tree feeds the fungi with photoassimilates to maintain the ECM symbiosis (Smith and Read, 2008). On the other hand, the saprotrophic fungi are principal decomposers in forests primarily obtaining the energy by degrading the soil organic matter (Baldrian *et al.*, 2011; Martin *et al.*, 2016). However, the role of ECM fungi in organic matter decomposition is becoming increasingly recognized (Kohler *et al.*, 2015), and it has been even proposed that their access to the host carbon facilitates the co-metabolic degradation of recalcitrant organic complexes mobilizing N from organic pools (Lindahl and Tunlid, 2015). The factors and mechanisms regulating spatial and temporal partitioning of these important fungal guilds are not yet well understood (Bödeker *et al.*, 2016; Peay, 2016), although they have critical importance for nutrient cycling in forest ecosystems.

Pinus pinaster Ait. is a characteristic and economically important pine species in the Mediterranean Basin. Maritime pines display great variability, and three main genetic pools corresponding to three geographic provenances, i.e. Atlantic, Mediterranean and African, have been distinguished (Alía and Moro, 1996; González-Martínez *et al.*, 2004; Rodríguez-Quilón *et al.*, 2016). These different pine genotypes mirror different phenotypes in characters as growth and biomass production, and/or fire and drought tolerance (Alía and Moro, 1996; González-Martínez *et al.*, 2004). Trees, both at intra or inter species level, can influence their associated soil fungal communities through the variation in the quantity and quality of the organic inputs they supply (Priha *et al.*, 1999; Kernaghan *et al.*, 2003; Aponte *et al.*, 2010; Bödeker *et al.*, 2016; Lamit *et al.*, 2016). This may potentially affect the functionality of the ecosystem, i.e., litter decomposition and nutrient cycling (Lane, 1991; Pregitzer *et al.*, 2013), although the processes and mechanisms involved are poorly understood. Because microbial extracellular enzymes are main responsible for the decomposition of complex organic matter compounds, they provide a useful assessment of the functional responses mediated by microbial and host nutrient demands (Olander and Vitousek, 2000; Allison and Vitousek, 2005).

In previous Chapters, we have demonstrated that among the strong spatial-temporal effects (i.e. site, season), the tree genotype emerges as a fundamental factor modulating the surrounding edaphic environment (Chapter 1), including the soil fungal communities (Chapter 2), and

structuring its associated rhizospheric microbial cloud, i.e., bacteria and ectomycorrhizal fungi (Chapter 3), and that these effects entail functional consequences. Given that the quality of carbon compounds differentially affects the decomposition by microorganisms (Waldrop and Firestone, 2004; Talbot *et al.*, 2012; Wang *et al.*, 2016), in this study we go further predicting that the soil quality generated under different tree genotypes and at different seasons will determine not only the taxonomic richness, but also the phylogenetic diversity of soil fungal communities. Given their distinctive and relative roles in organic matter decomposition (Kohler *et al.*, 2015) and nutrient cycling (Talbot *et al.*, 2013), a differential phylodiversity response of ectomycorrhizal and saprotrophic fungal guilds is also expected.

We have previously observed that soil fungal richness (i.e., α-diversity) can explain nutrient cycling processes, and a correspondence of taxonomic fungal guilds within functional ones (i.e., basidiomycetes and cellulose-degrading ascomycetes seemed to be mostly ectomycorrhizal) can exist (Chapter 2). We aim now to define which phylogenetic groups within the ectomycorrhizal and saprotrophic fungal guilds are responsible for which specific soil enzymatic functions affecting relevant ecosystem processes (i.e., C turnover, nitrogen and phosphorous mobilization). In a novel approach, we have combined here soil infrared spectral analyses together with phylogenetic methods and soil enzymatic tests to elucidate the mechanisms responsible for the impact of the tree genotype structuring soil fungal communities, and the derived functional consequences.

MATERIALS AND METHODS

Sampling and molecular analysis

The study was conducted in three ~45 year-old common garden plantations originally settled in a completely randomized block design with four blocks and trees of *Pinus pinaster* from different geographic origins (Alía and Moro, 1996). Sites were located in central Spain: Cabañeros (39º 22'N, 4º 24'W), Riofrío (39º 8'N, 4º 32'W), and Espinoso del Rey (39º 36'N, 4º 48'W) (see Chapters 1-3 for more details). The genetic and phenotypic variation of trees from the different geographic provenances selected for this study, i.e. Atlantic (Galicia, Spain), Mediterranean (Valencia, Spain), and African (Jbel Tassali, Morocco), has been previously demonstrated (Alía and Moro, 1996; Rodríguez-Quilón *et al.*, 2016). Soil sampling was carried out in spring and autumn 2012, and genomic DNA extracted as previously described (see Chapter 2 for a full description). The internal transcribed spacer region ITS1 of nuclear rDNA was amplified with the fungal primers ITS1F-ITS2, and two equimolar amplicon libraries (spring and autumn) were prepared for pyrosequencing (GsFLX-454 system, Roche Applied Biosystems, USA). Bioinformatics analyses were run, molecular operational taxonomic units defined

(MOTUs, at 97 % similarity) and taxonomically assigned with the UNITE database v.7.0 (Kõljalg *et al.*, 2013) (all details in Chapter 2). Fungal taxonomic assignment allowed classifying fungal MOTUs by life style i.e., ectomycorrhizal and saprotrophic fungi (Tedersoo and Smith, 2013; Tedersoo *et al.*, 2014; Nguyen *et al.*, 2016). Raw data were deposited in the Sequence Read Archive (SRA-NCBI, http://www.ncbi.nlm nih.gov/sra) as SRP076022.

Phylogeny reconstruction

As explained in Chapter 3, fungal phylogeny was approximated with the software Phylomatic as implemented in Phylocom v.4.2 (Webb *et al.*, 2008) and BEAST v.1.5.4 (Drummond and Rambaut, 2007). First, a phylogenetic fungal "mega-tree" was built by estimating the relationships among distantly related taxa within the fungal kingdom and the age for major nodes, using the phylogenetic information available in the literature (Table S1). Then, each fungal MOTU was matched to the most resolved position into the 'mega-tree', by using Phylomatic. If any fungal group (e.g. a genus) was missing in the mega-tree, the program returned a polytomy within the previous hierarchic group (i.e. polytomy of genera within the corresponding family) (Moles *et al.*, 2005). The ages of the remaining undated nodes were estimated with the BLADJ algorithm in Phylocom, which evenly distributes these nodes among others of known ages (Webb *et al.*, 2008). To test the robustness of our results to the topological and chronological uncertainty introduced by the Phylomatic + BLADJ procedures, we built another phylogenetic tree using the software BEAST v.1.5.4. This tree was based on a branch length adjustment procedure that follows a birth-death evolutionary model and polytomies are randomly resolved by BEAST (Drummond and Rambaut, 2007; Kuhn *et al.*, 2011). Markov Chain Monte Carlo (MCMC) analyses were run for 5 x 10^6 iterations, sampling trees every 10^3 iterations, discarding those trees at 25% burnin and recovering the maximum clade credibility tree with the TreeAnnotator v1.5.4 software (Drummond and Rambaut, 2007).

Phylogenetic structure of the fungal community

The phylogenetic structure of the fungal community was defined by using two phylogeny-weighted metrics on the constructed trees: the Net Relatedness Index (NRI) and the fuzzy-weighting method (PCPS) proposed by Pillar & Duarte (2010). The abundance-weighted mean pairwise phylogenetic distance (MPD) was first quantified with the *picante* R package (Kembel *et al.*, 2010), and then the Net Relatedness Index (NRI) was used as a standardized measure of MPD, calculated as NRI = (MPDobs − MPDrand) / sd(MPDrand), where MPDobs is the average of all pairwise phylogenetic distances between the taxa in a local community, MPDrand and sd(MPDrand) are the average and the standard deviations after randomly shuffling all taxa across the phylogenetic tree (Webb *et al.*, 2002). Additionally, the phylogenetic fuzzy-weighting method

was used to calculate the matrix P, using the *PCPS* R package (Debastiani *et al.*, 2015). This method describes the species phylogenetic composition of a plot considering the phylogenetic neighbourhood of each MOTU (see Chapter 3). To obtain the matrix P, a pairwise phylogenetic distance matrix was calculated and transformed to similarities between MOTUs for weighting the species composition matrix by a fuzzy set algorithm (Pillar and Duarte, 2010). In matrix P, each fungal MOTU has a value per plot that increases as the phylogenetic distance between neighbouring MOTUs decreases. To reduce the matrix P dimensionality, Principal Coordinate Analysis (PCoA) with Euclidean distances was used and scores along the first axis (PCPS1) were extracted. The maximal phylogenetic divergences among lineages is captured by this axis (Duarte *et al.*, 2012), which can be further used as proxy of the phylogenetic community structure (Chapter 3; Pérez-Valera *et al.* 2015). We calculated the contribution of each fungal phylum (mean ± SE) as the loadings of each taxon to the PCPS1. We performed Pearson's correlations between phylogenetic metrics based on the trees approached by Phylocom and BEAST to check for the consistency of our results. We yielded correlation values of 0.999, confirming the robustness of the analysis and a single tree was used in further analyses for simplicity.

Edaphic properties and ecosystem functioning

Given that soil quality can be precisely predicted using infrared technics (Joffre *et al.*, 2001), the near-infrared (5498 to 2198 cm^{-1}, NIR Overlapping Region A) scanning was used to approximate soil quality. The NIR spectrum was obtained by measuring soils with a HTS-XT Bruker spectrometer (Vertex 70, NIR-MIR-MCT, Bruker Corporation, Billerica, MA), as previously described (Chapter 1).

Extracellular enzymes are considered good proxies of soil functioning driving decomposition and nutrient cycling in soils (Sinsabaugh *et al.*, 2008). We evaluated the ecosystem functioning by measuring the activity of eight exoenzymes secreted by fungi following the methodology adapted from Mathieu *et al.* (2013): β-glucosidase (EC 3.2.1.3) and cellobiohydrolase (EC 3.2.1.91), implicated in cellulose degradation; xylosidase (EC 3.2.1.37) and β-glucuronidase (EC 3.2.1.31), involved in hemicellulose degradation; phosphatase acid (EC 3.1.3.2) mobilizing phosphorous; chitinase (EC 3.2.1.14) and L-leucineaminopeptidase (3.4.11.1) involved in nitrogen mobilization, and Lacasse (1.10.3.2), degrading recalcitrant compounds such lignin (see Chapters 2-3 for more details).

Statistical analyses

Variables were tested for normality (Shapiro test) and homoscedasticity (Levene test) and log or square root transformed when needed.

To determine whether the tree genotype and the season had an effect on the phylogenetic structure of soil fungal communities, we ran General Linear Mixed Models (GLMMs). The phylodiversity indices NRI and PCPS, indicative of the phylogenetic fungal community structure, were introduced as dependent variables, the tree genotype and the season as fix factors, and the site as random factor, in the models (p<0.05). Separate GLMM analyses per fungal life-style, e.g. ectomycorrhizal (ECM) and saprotrophic (SAP), were conducted.

To reduce the matrix dimensionality, data of the Near Infrared soil (NIRs) spectrum were analyzed by principal component analyses (PCA) with the function *dudi.pca* in the ade4 R package, as previously described (Chapter 1). The three first NIRs principal components explained the 64.1 % of the total variance. Season and tree genotype effects were significantly and respectively separated by PCA Axes 1 and 2 (Chapter 1), which were used for further modelling analyses.All these analyses were performed using the software R 3.1.1 (R Core Team, 2014).

To investigate the direct and indirect effects of the edaphic properties and the structure of the fungal functional groups (i.e. ectomycorrhizal and saprotrophs) on the ecosystem functioning, we performed Structural Equation Modelling (SEMs) with AMOS v.20.0 software (IBM Corporation Software Group, Somers, NY), based on an aprioristic model (Figure S1). Standardized path coefficients were estimated with the maximum likelihood algorithm (Shipley, 2002), and model fits evaluated according to the goodness-of-fit χ^2, the root mean square error of approximation (RMSEA), and additionally by the goodness-of-fit index (GFI) and the Bentler and Bonett's normed-fit index (NFI) (see Chapter 2). Among the different causal models tested, the best-fit one was chosen. Because, all *Pinus pinaster* were planted at the same period, the diameter at breast height (DBH) was used as surrogate of tree productivity. Soil quality was predicted by Near Infrared analysis, and the first and second principal PCA components of the NIRs overlapping Region A soil spectrum were used as proxies for the factors season and tree genotype, respectively (Figure S2) (see Chapter 1 for details). Negative tree genotype NIRs values indicated a prevalent effect of Mediterranean and African trees on soil quality, and positive NIRs values of Atlantic trees (Figure S2). Positive NIRs season values indicated a prevalent autumn effect on soil quality and negative NIRs values a main spring effect (Figure S2). The phylodiversity index PCPS1 was used as indicator of the phylogenetic structure of ectomycorrrhizal and saprotrophic fungal communities (Figure 1). The enzymatic processes chosen were representative of different nutrient cycles, i.e. carbon cycle (glucosidase, cellobiohydrolase, xylosidase, glucuronidase, laccase), nitrogen cycle (leucine and chitinase) and phosphorous cycle (acid phosphatase); and were evaluated in separated models. It was hypothesized that the productivity of trees, the tree genotype (Axis2, PCA-NIRs) and the season (Axis1, PCA-NIRs) would influence the ecosystem functioning (i.e., enzymatic processes), directly and through modulating the phylogenetic

structure of soil fungal communities (Figure S1). A differential response of the fungal functional guilds i.e., ectomycorrhizal and saprotrophic was expected.

RESULTS

A similar number of ectomycorrhizal and saprotrophic fungal MOTUs was identified in soil, i.e., 369 ECM (65 ± 11 per sample) and 392 SAP (66 ± 14 per sample), representing the 93 % of reads (Chapter 2). Among the ECM fungi, the 11.4% were Ascomycota and the 88.6 % Basidiomycota, while for the SAP the 51.3 % were Ascomycota, the 30.9 % Basidiomycota and the 17.8 % Zygomycota.

Phylogenetic structure of soil fungal communities

The phylogenetic index NRI revealed divergent structural patterns in the ectomycorrhizal (i.e., clustering, phylogenetically similar) and saprotrophic (i.e., over-dispersion, phylogenetically dissimilar) soil fungal communities (Figure S3a). The soil fungal community was similarly phylodiverse (NRI) under all tree genotypes (Figure S3b), while it was more phylodiverse in autumn than spring (Figure S3c).

Concerning the PCPS index, in the case of the ECM fungal community, positive values corresponded to Ascomycetes and negative to Basidiomycetes (see taxon loadings in Figure 1a). For the saprotrophic community, negative PCPS1 corresponded to Ascomycetes and Basidiomycetes and positive to Zygomycetes (Figure 1a), and when these last were excluded, a pattern inverse to that of ECM was observed (Figure 1a). For both ECM and SAP fungal guilds, Basidiomycetes tended to co-occur with closer relatives (i.e., lower matrix P values) compared with Ascomycetes that mainly co-existed with evolutionarily distant lineages (i.e., higher matrix P values) (Figure S4). The ECM pattern was opposite to that found in the rhizosphere (Chapter 3).

When the first principal component of the phylogenetic community structure (PCPS1) was modelled, a significant effect of the tree genotype on its associated soil ECM fungal community was revealed (Figure 1b; Table S2), with the prevalence of Ascomycetes in soils under the Atlantic trees, and of Basidiomycetes in soils under the Mediterranean genotype (Figure 1b). In the case of the soil SAP community, the tree genotype effect was detected only when Zygomycetes were excluded from the analysis (Figure 1b), with Basidiomycetes dominating in soils under the Atlantic genotype and Ascomycetes in soils under the Mediterranean trees (Figure 1b), opposite to that observed for ECM.

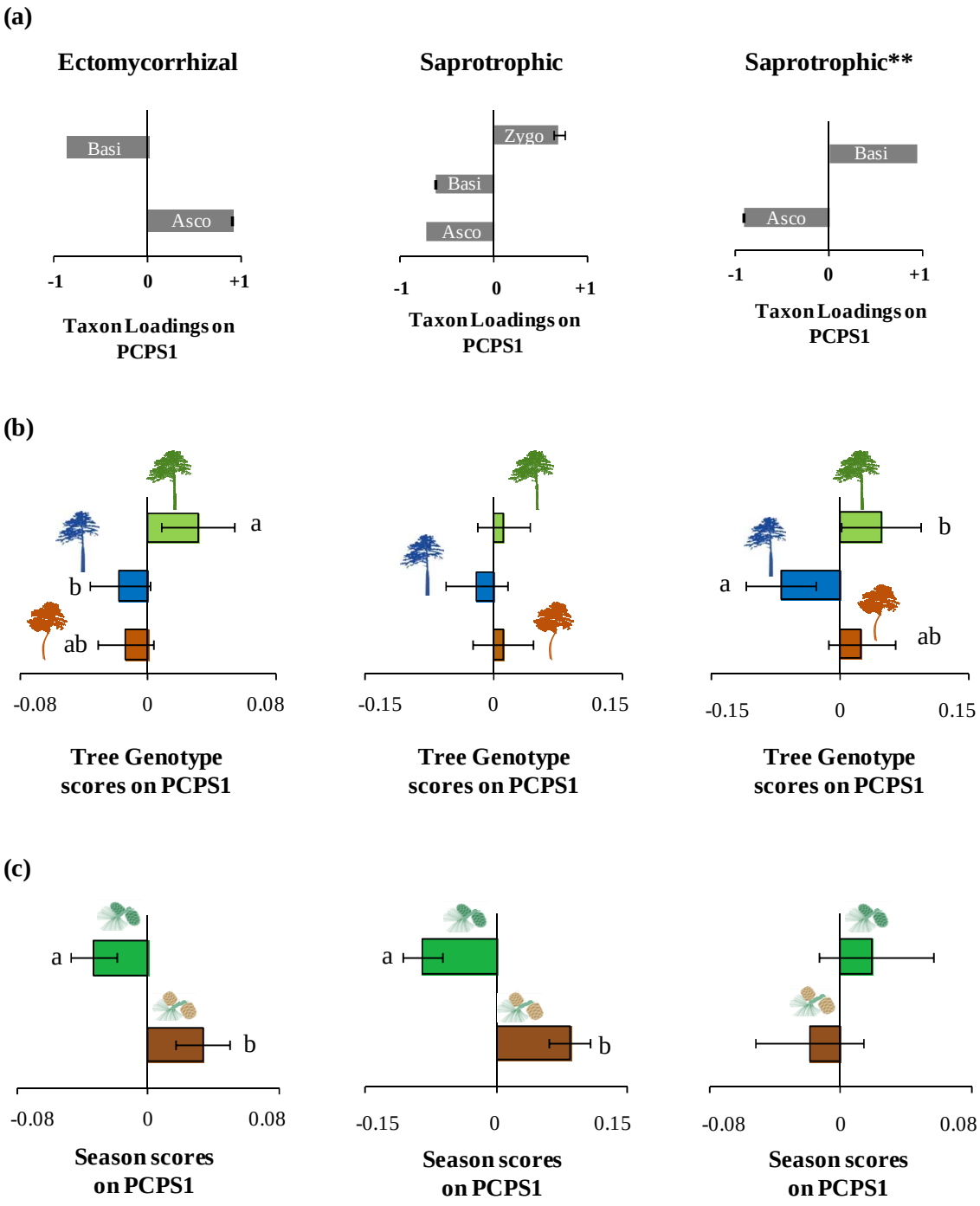

Figure 1 | (a) Loadings (means ± SE) of each taxon on the first principal component (PCPS1) of the phylogenetic structure of fungal communities by life-style (ECM=ectomycorrhizal, SAP=saprotrophic and SAP**=saprotrophic without Zygomycetes), (b) scores of tree genotype on PCPS1 by life-style and (c) scores of season on PCPS1 by life-style. The PCPS1 explained the 41% (ECM), the 36 % (SAP) and the 48 % (SAP**) of the total variance respectively. Grey bars indicate the phyla dominance while bar colours indicate different tree genotypes (light green= Atlantic; blue= Mediterranean, orange= African) and season (dark green= spring; brown = autumn). Within each graph, different letters denote significant differences among genotype and/or seasons according to the Tukey test ($p<0.05$).

A clear seasonal effect was detected on the phylogenetic structure of soil fungal communities (Figure 1c; Table S2). In the case of ECM fungi, Basidiomycetes dominated in spring and Ascomycetes in autumn (Figure 1c), while for SAP, a significant preponderance of Basidiomycetes and Ascomycetes was observed in spring, while Zygomycetes prevailed in autumn (Figure 1c).

Relationships between factors, fungal phylodiversity and ecosystem functioning

The structural-equation model proposed for evaluating the ecosystem functioning, provided a good fit for all enzymes, as indicated by the non-significant f value (χ^2 = 1.39; p = 0.499) and by the goodness of fit indices (RMSEA<0.001; NFI and GFI>0.97) (Figure 2).

The best-fitted structural-equation models indicated different functional patterns for the ectomycorrhizal and saprotrophic fungal communities in soil (Figure 2). Through modifying the edaphic environment, the tree genotype and the season regulated the phylogenetic structure of soil ECM and SAP fungal communities (Figure 2).

Tree genotype and season effects on soil ECM and SAP communities were coincident with those previously observed in Figure 1, indicating a good representativeness of the principal components of the near infrared analysis as proxies of factor-mediated effects on the soil quality. The tree productivity marginally affected the phylogenetic structure of the ECM fungal community (i.e., higher DBH associated with Basidiomycetes, according to results in Chapter 2) (Figure 2b).

In the case of the ECM fungal community (Figure 2a), activities related to N and P mobilization were directly explained by the tree genotype (i.e., higher leucine activity under Atlantic trees) and the season (i.e., higher phosphatase and chitinase activities in spring). Furthermore, a N-cycling process was dependent on the ECM phylodiversity, with ECM Ascomycetes (i.e., prevalent under the Atlantic genotype and in autumn, Figure 1a) marginally explained the chitinase activity (Figure 2a). Regarding the carbon cycle (Figure 2a), the cellulose-degrading enzymes were highly responsive to the productivity of trees, and the tree genotype directly explained the laccase activity (i.e., higher under Mediterranean and African trees). Soil ECM Ascomycetes (i.e., prevalent under the Atlantic trees and in autumn, Figure 1a) were the main responsible for laccase and cellulose and hemicellulose-degrading activities (Figure 2a).

In the case of the SAP fungal community (Figure 2b), the tree genotype (i.e., lower laccase and higher leucine activity under Atlantic trees), as well as the season (i.e., higher phosphatase and chitinase activity in spring, and hemicellulose degrading activity in autumn), directly explained activities related with the C turnover and the mobilization of nutrients (Figure 2b). Among these functions, N mobilization and C turnover were dependent on the phylogenetic

structure of the saprotrophic fungal community, with SAP Basidiomycetes (i.e., prevalent under the Atlantic genotype and in spring, Figure 1b) explaining the leucine activity (Figure 2b), and SAP Ascomycetes (i.e., prevalent under the Mediterranean and African genotypes and in autumn, Figure 1b) explaining cellulose-degrading activities (Figure 2b).

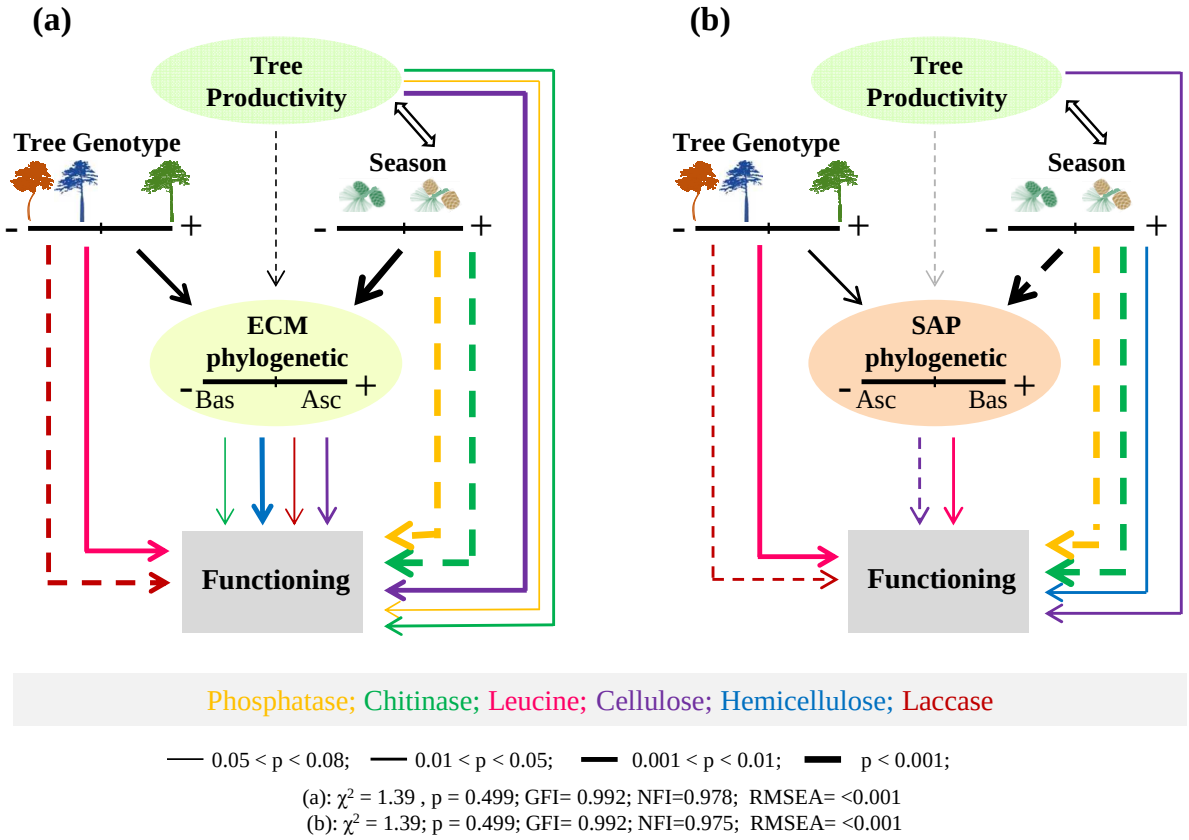

Figure 2 | Structural equation models representing causal relationships among the influence of tree productivity (diameter at breast height, DBH), the tree genotype (PCA-Axis2 of the Near Infrared Soil analysis), season (PCA-Axis1 of the Near Infrared Soil analysis) and fungal phylogenetic structure (PCA-Axis1 of the PCPS matrix) on the ecosystem functioning. (a) Ectomycorrhizal (ECM) and (b) Saprotrophic (SAP) fungal communities. Arrows indicate causal relationships: positive effects by solid lines, and negative effects by dashed lines. Significant correlations are indicated with double arrows. Different colours of arrows depict the hypothesized model for each enzymatic activity. Arrow widths are proportional to P values. DBH correlation with tree genotype and season was taken into account to fit the model. Paths with coefficients non-significant different from 0 (p>0.08) are shown in grey. χ^2, p-value and fit statistics (NFI, GFI and RMSEA) of each model are also indicated.

DISCUSSION

Our replicated common garden study that recovered seasonal variability and intra/inter site conditions, demonstrates that adult tree genotypes, similar to engineering organisms, can modify key ecosystem services by direct variation of the soil quality and through modulating the phylogenetic structure of its soil mycobiome. Besides, our results evidence different mechanistic patterns for the main ecological fungal guilds in forest soils, i.e., ectomycorrhizal and saprotrophic, for which specific phylogenetic clades have been identified associated with concrete ecosystem processes.

Phylogenetic structure of soil fungal communities

Compared with the ECM fungal community, soil saprotrophs showed an over-dispersed phylogenetic pattern, likely defined by the distant Zygomycetes phylum. Similar to what happens in plant communities, co-occurrence of fellow fungal clade members can be promoted by similarity of habitat use, which in turn could predict increased competition pressure (Prinzing *et al.*, 2017), although evidences reported for fungal communities are unclear (Bahram *et al.*, 2015; Moeller and Peay, 2016; Peay, 2016).

The phylogenetic metric PCPS1 gave us a deeper insight than that previously obtained through the analysis of local and regional fungal taxonomic richness (i.e., α-β-diversity; Chapter 2), by revealing the relative weight of representative phylogenetic clades within each ecological fungal guild (i.e., ectomycorrhizal-ECM and saprotrophic-SAP). These results highlight the importance to include phylogenetic metrics in fungal community studies, since functional traits tend to be similar between related species (Talbot *et al.*, 2015; Treseder and Lennon, 2015; Goberna and Verdú, 2016; Pena *et al.*, 2016). Our study showed that, similar to SAP, the soil ECM Basidiomycetes tended to co-occur with closer relatives, while Ascomycetes mainly co-existed with evolutionarily distant lineages. Remarkably, the opposite pattern was observed for the ECM fungal community in the rhizosphere (i.e., ECM fungi associated with root-tips of the same trees) (Chapter 3), indicating a strong spatial partitioning for this fungal guild. Different assembling forces could be unequally acting at each edaphic compartment (rhizosphere and soil), with priority effects, substrate-mediated niche separation and/or competitive exclusion among the main mechanisms reported driving ECM assemblages (Kennedy *et al.*, 2009; Bödeker *et al.*, 2016; Moeller and Peay, 2016).

Tree genotype and seasonal effects on soil fungal communities

As hypothesized, the *P. pinaster* genotype exerted a clear effect on the phylogenetic structure of soil fungal communities, and this effect was different depending on the ecological fungal guild. The phylogenetic assemblage of ECM fungi in soil was significantly modulated by

the tree genotype, with ECM Basidiomycetes preferentially represented under the higher productive Mediterranean trees, and an overrepresentation of ECM Ascomycetes under the Atlantic trees (see Chapters 2-3). The carbon and nitrogen demand of Basidiomycetes, being able to form large rhizomorphs, is thought to be higher than that of Ascomycetes (Agerer, 2001; Tedersoo *et al.*, 2006). In our study, ECM Basidiomycetes could be favoured by greater organic inputs supplied by high productive tree genotypes, whereas Ascomycetes would relate with more nutrient limiting conditions. Linkages between the prevalence of Basidiomycetes or Ascomycetes depending on tree growth (Korkama *et al.*, 2007) and photosynthetic activity (Brown et al., 2001; Mueller and Gehring, 2006) have been previously reported. Interestingly, we previously saw in Chapter 3 the same Basidiomycetes and Ascomycetes genotype preferences for rhizospheric ECM fungi.

Likewise, the tree genotype determined the phylogenetic structure of the soil saprotrophic community, but with an inverse effect than that observed for ECM, i.e., saprotrophic Basidiomycetes overrepresented under the Atlantic genotype, and Ascomycetes under the Mediterranean trees. Different niche occupation and/or competition are possible mechanisms explaining these opposite patterns. In the first case, soil quality variations due to the tree genotype influence, i.e., probably linked to different composition/recalcitrance of organic inputs, might select specific saprotrophic or ectomycorrhizal fungal communities (Rajala *et al.*, 2010; Bödeker *et al.*, 2016; Uroz *et al.*, 2016). On the other hand, concerning competition, soil ECM and SAP fungal mycelia interact each other modifying their respective growth rates during the decomposition process (Leake *et al.*, 2001; Bending, 2003; Lindahl and Boberg, 2008; Fernandez and Kennedy, 2016). Competition has been explained by structural and functional similarities between fungal guilds, common nutrient requirements and mycelia scavenging in the same settings (Leake *et al.* 2002). This competitive interaction could be especially enhanced under the restrictive environmental conditions imposed by the Mediterranean climate, i.e., with poorly developed soils and limited nutrient and water availability, as previously signalled (Fernandez and Kennedy, 2016). In line with this, Koide and Wu (2003) showed that the ability of ECM to compete with SAP depended on the soil moisture, and a plausible mechanism explaining it is that ECM fungi can acquire water from their hosts during drought periods via hydraulic redistribution (Querejeta *et al.*, 2003). Additionally, our results led us to think that this competitive interaction between ecological fungal guilds also happened at the level of big phyla, probably more pronouncedly for Basidiomycota that is a phylum with abundant long exploration type species forming large mycelial cords and rhizomorphs in soil (Boddy, 1993).

In previous Chapter 2, α- and β-diversity indices revealed a different seasonal pattern for ECM and SAP fungi. Now, the phylogenetic structure of these communities allowed ascribing the

proliferation of ECM in spring to an overrepresentation of closely related Basidiomycetes and the SAP increase in autumn to the predominance of Zygomycetes, which tended to co-exists with closer relatives. The first case could be related with a higher C allocation belowground by more active trees in spring (Kaiser *et al.*, 2010) and to a change in resource availability as water and nutrient content (Voříšková *et al.*, 2014). By contrast, Zygomycetes probably were more responsive to changes in temperature, moisture, and nutrient availability (Allison and Treseder, 2008).

Convergence of tree genotype, fungal community and ecosystem functioning

Our results illustrate that the quality of soils generated by abiotic (i.e., seasonal influence) and biotic (i.e., organic inputs of different tree genotypes) factors regulates relevant ecosystem services by two principal routes, through variations in the phylogenetic structure of soil fungal communities, and directly and independently of them. Additionally, a great portion of the variability generated by each factor on soil properties was recovered by near infrared spectral analysis and hence, NIRs spectrum emerges as a good proxy for soil quality. Our results agree with the fact that the C:N:P stoichiometry and the organic matter concentrations are major universal factors influencing soil enzymatic activities at different spatial scales (Sinsabaugh *et al.*, 2008; Kivlin and Treseder, 2014; Courty *et al.*, 2016). Fungal communities would be regulated not only by the carbohydrates directly provided by trees but also by external delivery of complicated carbon polymers from litter (Aučina *et al.*, 2007; Aponte *et al.*, 2010; Velmala *et al.*, 2013; Uroz *et al.*, 2016). Additionally, other microbial groups could be operating.

Our results also showed that specific enzymatic activities could be attributed to particular phylogenetic fungal clades. For saprotrophs, an overrepresentation of Basidiomycetes, mainly associated with Atlantic trees in spring, predicted higher N mobilization, while Ascomycetes related to cellulose degradation. Moreover, soil ECM Ascomycetes were mainly involved in the C turnover, being favored under poor productive Atlantic trees and in autumn. Although the different ecology of big fungal phyla is acknowledged (Tedersoo *et al.*, 2006; Jones *et al.*, 2010), the related functional consequences are poorly understood. This is especially true for ECM Ascomycota, usually thriving in harsh conditions (e.g., Mediterranean ecosystems) and that, aside from organic matter degradation roles, can also invest energy in stress resistance related to components such as melanin (e.g., *Cenoccocum* spp.) (Iakovlev and Stenlid, 2000; Koide *et al.*, 2014). In this situation, different interactive mechanisms between trees and ECM fungal phyla, that is resistance to stress *vs.* nutrient acquisition, would operate (Treseder and Lennon, 2015; Moeller and Peay, 2016).

Although the phylogenetic structure of rhizospheric and soil ECM community was relatively similar, they explained different ecosystem functions (see Chapter 3), probably reflecting niche differentiation of ECM individuals. In fact, the ECM Basidiomycetes settled in root tips tended to co-exist with phylogenetically distant relatives whereas in soil they co-existed with more closely related. According to Courty *et al.* (2016) and Talbot *et al.* (2013), the contributions of ECM fungi to larger-scale soil C and nutrient cycling may occur primarily via extramatrical hyphae outside the rhizosphere. In fact, ca. 80% of the ECM biomass in forest soils corresponds to mycelial systems (Wallander *et al.*, 2001; Leake *et al.*, 2004), which are a strong sink of tree carbohydrates and an important extension of the root system for the absorption of nutrients (Cairney and Burke, 1996; Smith and Read, 2008).

In conclusion, our results demonstrate that adult tree genotypes are able to modify key ecosystem services by direct variation of the soil quality and through modulating the phylogenetic structure of its associated soil fungal communities. We identified the contributions of different ectomycorrhizal and saprotrophic phylogenetic clades to C turnover and nutrient mobilization. Consecutively, we suggest that these intricate relations may entail functional consequences for the entire ecosystem supporting the theory of the extended phenotype (Whitham *et al.*, 2003).

SUPPORTING INFORMATION

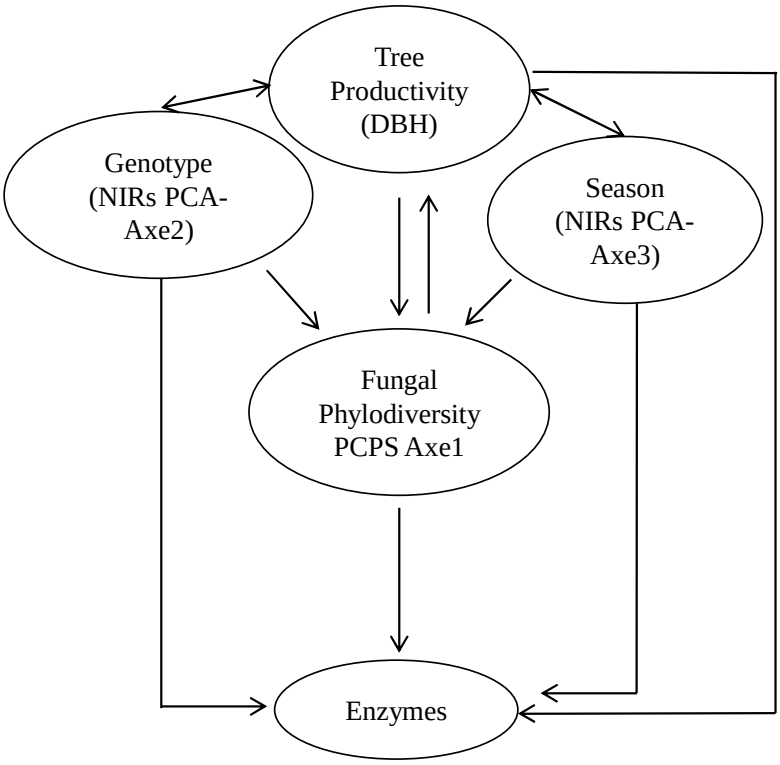

Figure S1 | Path diagram representing hypothesized causal relationships among the influence of tree productivity, genotype, season, fungal phylodiversity and ecosystem functioning. Arrows depict casual relationships. Correlations are indicated with double arrows.

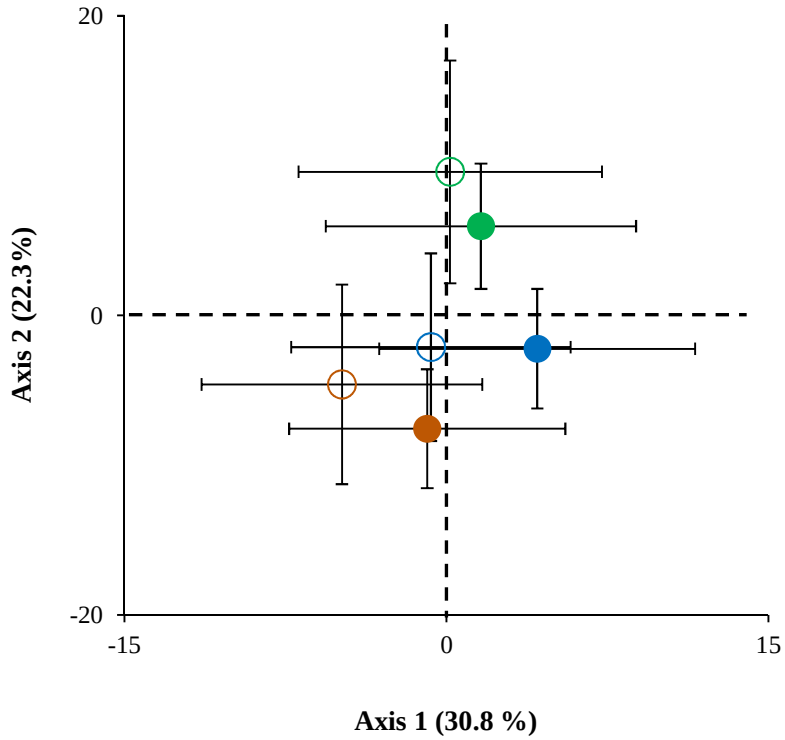

Figure S2 | Principal component analyses of near infrared (NIR) spectral data (corresponding to the region 5498 to 2198 cm^{-1}), by tree genotype (green = Atlantic; blue = Mediterranean, orange = African), and season (open = spring; filled = autumn). The explained variance by each axis is shown inside brackets. Taken from Chapter 1.

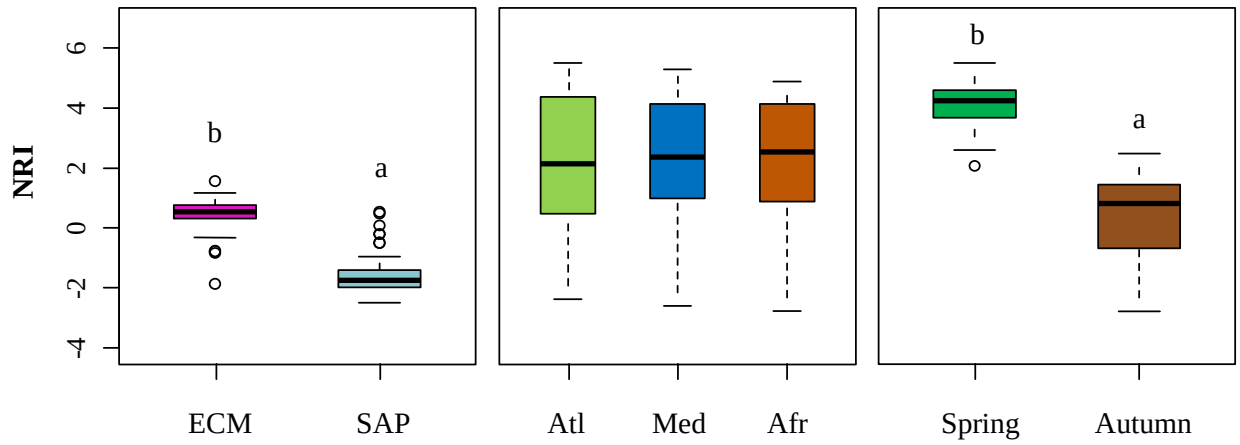

Figure S3 | Phylogenetic Net Relatedness Index (NRI) of total fungal community by (a) lifestyle (pink=ectomycorrhizal-ECM; blue=saprotrophs-SAP), (b) tree genotype (light green = Atlantic; blue = Mediterranean, orange = African) and (c) season (dark green = spring; brown = autumn). Boxes represent the interquartile range (IQR) between first and third quartiles and the horizontal line inside is the median. Whiskers denote the lowest and highest values within 1.5 x IQR from the first and third quartiles, respectively. Within each graph, different letters denote significant differences among treatments according to Tukey test ($p<0.05$).

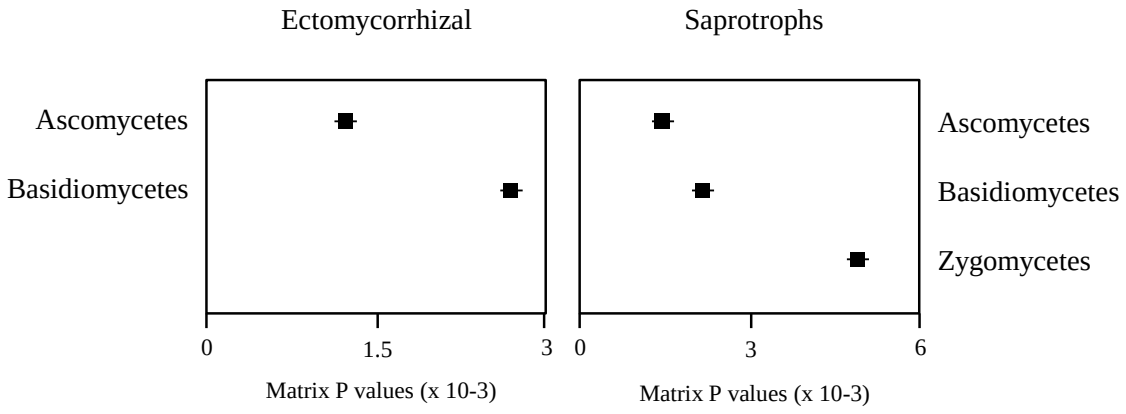

Figure S4 | Scores of main fungal phyla in matrix P, for ectomycorrhizal and saprotorophic fungal communities. Values are means ± SD. In matrix P, each MOTU has a value per sample that increases as the phylogenetic distance between neighbouring MOTUs decreases.

Table S1 | Bibliographic references used to infer the relationships among distantly related taxa within the Fungal Kingdom and the age for major nodes in the phylogenetic "megatree", which was used to study the phylogenetic structure of fungal communities.

Kingdom	Phylum	Subphylum	Class/Subclass	Order	Family	
Fungi						
Ebersberger *et al.* (2012)	**Basidiomycota**	**Agaricomycotina**	**Homobasiodiomycetes**			
Hibbet *et al.* (2007)	Matheny *et al.* (2007)	Hibett (2006)	Binder *et al.* (2005)			
James *et al.* (2006)		Hibbet *et al.* (2014)	Bodensteiner *et al.* (2004)			
Larsson *et al.* (2007)			Larsson *et al.* (2004)			
Tedersoo *et al.* (2010)			**Agaricomycetes**	**Agaricales**	**Agaricaceae**	Vellinga (2004; 2011)
Tehler *et al.* (2003)			Floudas *et al.* (2012)	Matheny *et al.* (2006)	**Clavariaceae**	Dentinger and McLaughlin (2006)
			Hibbet *et al.* (2014)		**Entolomataceae**	Baroni and Matheny (2011)
					Hygrophoraceae	Lodge *et al.* (2014)
					Inocybaceae	Alvarado *et al.* (2010)
					Lyophillaceae	Sanchez-García *et al.* (2014)
					Psathyrellaceae	Nagy *et al.* (2011)
					Tricholomataceae	Sanchez-García *et al.* (2014)
				Atheliales		
				Kotiranta *et al.* (2011)		
				Larsson *et al.* (2004)		
				Boletales	**Boletaceae**	Wu *et al.* (2014)
				Binder and Hibett (2006)		
				Wilson *et al.* (2012)		
				Cantharellales		
				Diederich *et al.* (2014)		
				Moncalvo *et al.* (2006)		
				Dacrymycetales		
				Kirschner *et al.* (2005)		
				Shirouzu *et al.* (2013)		
				Hymenochaetales		
				Larsoon *et al.* (2006)		
				Polyporales		
				Binder *et al.* (2013)		
				Larsoon (2007)		
				Russulales		
				Miller *et al.* (2006)		
				Sebacinales		
				Oberwinkler *et al.* (2014)		
				Selosse *et al.* (2009)		
				Thelephorales	**Thelephoraceae**	Tedersoo *et al.* (2014)
				Larsson *et al.* (2004)		
				Auriculariales		
				Sotome *et al.* (2014)		
				Weib and Oberwinkler (2001)		
				Zhou *et al.* (2013)		
			Phallomycetidae	**Phallales**		
			Giachini *et al.* (2010)	Hosaka *et al.* (2006)		
				Gomphales		
				Hosaka *et al.* (2006)		
			Tremellomycetes	**Cystofilobasidiales**		
			Millanes *et al.* (2011)	Fell and Scorzetti (2004)		
			Wallemiomycetes			
			Hibbet *et al.* (2014)			
		Puccinomycotina	**Mycrobotriomycetes**			
		Libkind *et al.* (2011)	Sampaio *et al.* (2003)			

Continuation **Table S1**

Kingdom	Phylum	Subphylum	Class/Subclass	Order	Family
	Ascomycota		**Archaeorhizomycetes**		
	Schoch *et al.* (2009)		Menkis *et al.* (2014)		
			Dothideomycetes	**Dothideales**	
			Bohem *et al.* (2009)	Bills *et al.* (2012)	
			Schoch *et al.* (2009)	**Botryosphaeriales**	
				Slippers *et al.* (2013)	
				Capnodiales	
				Crous *et al.* (2009)	
				Yang *et al.* (2014)	
				Pleosporales	**Pleosporaceae** Ariyawansa *et al.* (2015)
				Kodsueb *et al.* (2006)	**Venturiaceae** Crous *et al.* (2007); Machouart *et al.* (2014)
			Eurotiomycetes		**Trichocomaceae** Houbraken and Samson (2011)
			Chen *et al.* (2015)		
			Geiser *et al.* (2006)		
			Lecanoromycetes		
			Miadlikowska *et al.* (2006; 2014)		
			Leotiomycetes		
			Cai *et al.* (2009)		
			Gernandt *et al.* (2001)		
			Hambleton and Sigler (2005)		
			Hambleton *et al.* (2005)		
			Wang *et al.* (2006a,b)		
			Geoglossomycetes		
			Wang *et al.* (2006b)		
			Orbiliomycetes		
			Wang *et al.* (2006b)		
			Pezizomycetes		**Pyronemataceae** Hansen *et al.* (2013); Sbissi *et al.* (2010)
			Perry *et al.* (2007)		
			Sordariomycetes	**Sordariales**	**Lasiosphaeriaceae** Kruys *et al.* (2015)
			Maharachchikumbura *et al.* (2015)	Huhndorf *et al.* (2004)	
			Réblová *et al.* (2008)	**Xylariales**	
			Summerbell *et al.* (2011)	Asgari and Zare (2011)	
			Zhang *et al.* (2006)	Jaklitsch and Voglmayr (2012)	
				Hypocreales	**Nectriaceae** Lombard *et al.* (2015)
				Chaverri *et al.* (2011)	**Hypocreaceae** Kullnig-Gradinger *et al.* (2002)
				Gräfenhan *et al.* (2011)	
				Johnson *et al.* (2009)	
	Glomeromycota				
	Kruger *et al.* (2012)				
	Redecker and Raad (2006)				
	Zygomycota			**Mucorales**	
	Chang *et al.* (2015)			Vitale *et al.* (2011)	
	White *et al.* (2006)				
	Chytridiomycota				
	James *et al.* (2006)				
Node age datation	Amo de Paz *et al.* (2011)				
	Beimforde *et al.* (2014)				
	Berbee and Taylor (2010)				
	Chen *et al.* (2015)				
	Floudas (2012)				
	Hedges (2015)				
	Kohler *et al.* (2015)				
	Rouxel *et al.* (2011)				

Table S2 | Effects of tree genotype, season and its interaction on the phylogenetic structure measured by the Net Relatedness Index (NRI) and the fuzzy-weighting method (PCPS) of the ectomycorrhizal (ECM), saprotrophic (SAP) and saprotrophic without Zygomycetes (SAP**) fungal communities, analysed by General Linear Mixed Models with site as random factor (F values; .p<0.08*p<0.05;**p<0.01;***p<0.001).

		ECM		**SAP**		**SAP****	
	df	*NRI*	*PCPS1*	*NRI*	*PCPS1*	*NRI*	*PCPS1*
Genotype	2	1.05	**3.93***	0.22	0.40	1.30	**2.97***
Season	1	**20.21*****	**19.69*****	**6.26***	**28.88*****	**3.61.**	0.90
Gen x Sea	2	0.02	0.12	0.07	0.21	0.85	0.30

Chapter 5

The fire regime affects the quality and functioning of soils in Mediterranean pine forests

INTRODUCTION

Soil plays a critical role in the fertility and stability of forest ecosystems (Augusto *et al.*, 2002). Long-term pedogenesis processes are conditioned by multiple factors over the time, e.g., plant community composition, microorganisms, environmental conditions and/or disturbances. Microbial communities are a main component of forest soils driving fundamental processes for the ecosystem functioning (van der Heijden *et al.*, 2008). Particularly, microbial enzymatic activities are crucial for the ecosystem sustainability since they have been considered as the proximal driver of decomposition (Sinsabaugh *et al.*, 2008), influencing soil carbon storage and nutrient mobilization (Courty *et al.*, 2005; Sinsabaugh *et al.*, 2009). Extracellular enzymes hydrolyse complex compounds of organic matter mostly from plant-derived material and microbial residues (Baldrian, 2014). Soil quality and enzymatic activity are influenced by different abiotic and biotic factors (i.e., pH, climate, composition of organic matter and soil microbial community) (Sinsabaugh *et al.*, 2008; Baldrian *et al.*, 2010; Kivlin and Treseder, 2014; Courty *et al.*, 2016), and by environmental disturbances, as for example fire (Hernández *et al.*, 1997; Holden *et al.*, 2013). The recalcitrance of organic substrates, particularly the lignin and N contents in litter, influences the activity of extracellular enzymes and hence the decomposition rates (Fog, 1988; Sinsabaugh *et al.*, 2002; Theuerl and Buscot, 2010; Talbot *et al.*, 2012). Zhu and Wang (2011) have reported that the quality of soil organic matter (SOM) can regulate the allocation and stabilization of N in SOM. Thus, persistent soil disturbances, e.g., recurrent burning in Mediterranean fire-prone forests, may affect the litter and SOM quality, and hence the ecosystem functioning. In this sense, Hart et al. (2005) have established that, in the long-term, plant–soil feedbacks are more decisive to the maintenance and stability of fire-adapted forests than the direct nutrient mineralizing effect of fire. The management of soil systems requires diagnostic indicators to asses spatial and temporal ecosystem trends in response to changing disturbance regimes, e.g., recurrent fires (Cohen *et al.*, 2006; Cécillon *et al.*, 2009). Within this context, since infrared soil spectra reflect a group of soil components that can be analyzed simultaneously (Terhoeven-Urselmans *et al.*, 2006; Viscarra Rossel *et al.*, 2006; Ludwig *et al.*, 2008, 2015), the use of these techniques has been proposed as an integrative variable of soil quality (Cécillon *et al.*, 2009; Akroume *et al.*, 2016), as previously signaled (Chapter 1).

Fire triggers the physical and chemical degradation of soils (Gimeno-García *et al.*, 2000), and shapes the structure of soil microbial communities and their activity (Hart *et al.*, 2005; Cairney and Bastias, 2007; Rincón and Pueyo, 2010). Indirect fire effects include changes in the vegetation cover, which further contributes to the drastic reduction of its associated microbiota (Hart *et al.*, 2005; Buscardo *et al.*, 2015). Recurrent fire is a major intrinsic ecological factor shaping living communities in Mediterranean ecosystems (Keeley *et al.*, 2011). The dramatic

increase of wildfires frequency and intensity due to the recent rise of temperatures (Pausas, 2004) may be modifying the soil abiotic environment as well as the biotic component, and compromising the resilience of these ecosystems by altering their functioning.

Because fire is closely linked to Mediterranean ecosystems, the natural vegetation of this region has developed different mechanisms of adaptation to this disturbance. *Pinus pinaster* Ait. and *Pinus halepens*is Mill. (Tapias *et al.*, 2004) are two representative pine species distributed in the Mediterranean Basin and with contrasted ecology (Ruiz *et al.*, 2009), showing characteristic fire-adapted traits.

In this work, we studied soils from natural populations of *P. pinaster* and *P. halepensis* subjected to contrasted fire regimes (Hernández-Serrano *et al.*, 2013), by using infrared spectral approaches and enzymatic analyses with the aim to evaluate whether the contrasted soil environment between *P. pinaster* and *P. halepensis* forests conditioned the cycling of nutrients and if the fire regime determined the overall quality of soils further altering relevant ecosystem processes. Our hypotheses were that i) forests of different Mediterranean pine species would show different quality and functioning of soils, and that ii) historically recurrent fires in these fire-prone ecosystems would have left a signature in the overall quality of soils determining the cycling of nutrients.

MATERIAL AND METHODS

Site description and experimental design

The study was conducted in natural forests of *P. pinaster* (Ppi) and *P. halepensis* (Pha) located in eastern Spain and characterized by Mediterranean climate (Figure S1). The plant community was dominated either by maritime or Aleppo pine. In the case of *P. pinaster* forests, the under-storey was composed of scattered *Quercus suber* L., *Quercus coccifera* L., *Lavandula stoechas* L., *Cystus* sp., *Ulex parviflorus* Pourret, *Amelanchier ovalis* Medik. For *P. halepensis* forests, the under-storey was composed of several evergreen shrubs, e.g. *Q. coccifera*, *Erica arborea* L., *Cystus* sp., *U. parviflorus*, *Juniperus* sp., *Rosmarinus officinalis* L., and *Pistacia lentiscus* L.

Seven populations of *P. pinaster* and eight of *P. halepensis* were selected in locations with different fire regimes (Hernández-Serrano *et al.*, 2013) (Figure S1). Nine populations are located in areas where crown-fires are historically frequent and most regeneration events are driven by fire (HiFi populations), and the remaining six populations are located in areas where crown-fires are rare and most regeneration events are independent of fire (LoFi populations) (Verdú and Pausas, 2007) (Figure S1). In the study area, fire is strongly linked to drought and recent fire history information shows that more than 50% of the area in HiFi burned at least once during the

1978–2001 period (Pausas *et al.*, 2004; Pausas and Fernández-Muñoz, 2012), while in LoFi, the proportion was under 15% (Abdel Malak and Pausas, 2006). Soil fertility in these forests is linked to the bedrock type, with all *P. pinaster* populations settled on siliceous soils, while HiFi and LoFi sites of *P. halepensis* include both siliceous and calcareous soils (Ojeda *et al.*, 2010) (see details in the next Chapters).

Soil sampling and physical-chemical analyses

Sampling was carried out in spring 2013. In each pine population, five soil samples separated of more than 10 m were collected (n = 75). At each sampling point, the litter was removed and soil samples were obtained by excavating 10 x 10 x 20 cm holes at N, S, E and W orientations in a grid of 1 m^2. These subsamples were pooled and kept at 4 ºC in plastic bags until processing. Soil aliquots were collected and kept fresh at 4 ºC for immediate enzymatic measurements. Remaining soil was air dried and sieved (2 mm) for subsequent analyses.

The relative humidity (RH) of soil samples was determined by drying at 65 ºC for 48 h. Air-dried soils were measured for pH (1:5, w:v in H_2O), electrical conductivity (1:5, w:v in H_2O), organic matter (OM) (Walkley and Black, 1934), total N (Kjeldahl method), and extractable P and K determined by inductively coupled plasma spectrometry (Optima 4300DV, Perkin-Elmer). Furthermore, mid and near infrared spectroscopic soil analyses (MIR and NIR) were conducted to obtain an integrative proxy of the soil quality (Cécillon *et al.*, 2009). Prior to performing MIR-NIR analyses, soil samples were re-dried at 30 °C for 24 h. Three replicates per sample of 20-30 mg soil were analyzed by mid-infrared (5498 to 549 cm^{-1}, MIR) and near-infrared (9997 to 2198 cm^{-1}, NIR) scanning using a HTS-XT Bruker spectrometer (Vertex 70, NIR-MIR-MCT, Bruker Corporation, Billerica, MA), the two sensors overlapping in the 5498-2198 cm^{-1} region. The spectra were recorded at intervals of 1.9 cm^{-1}, and the full range provided by each sensor was measured.

Enzymatic tests

A total of eight enzymatic assays were performed on fresh soils using the method adapted from Mathieu et al., (2013). Briefly, seven enzymatic tests were based on fluorogenic substrate release, methylumbelliferone (MU) or methylcoumarine (AMC), upon cleavage by enzymes: MU-β-d-glucopyranoside (MU-G) for β-glucosidase (EC 3.2.1.3), MU- β-D-cellobioside (MU-C) for cellobiohydrolase (EC 3.2.1.91), MU-phosphate free acid (MU-P) for phosphatase acid (EC 3.1.3.2), MU-N-acetyl-β-glucosaminide (MU-Q) for chitinase (EC 3.2.1.14), MU- β-D-xylopyranoside (MU-X) for xylosidase (EC 3.2.1.37), 4-MU-β-D-glucuronide hydrate (MU-GU) for β-glucuronidase (EC 3.2.1.31), and L-leucine-7-AMC (AMC-L) for L-leucineaminopeptidase (3.4.11.1) (Sigma Aldrich Chemicals, Madrid, Spain). All enzymatic assays were done in 96-well

microplates including controls performed by heating soil suspensions at 100 ºC for 10 min. Reactions were performed at room temperature in the dark with different stirring incubation times depending on the enzyme. Measurements were carried out with a Victor microplate reader (Perkin-Elmer Life Sciences, Massachusetts, USA), with 355/460 nm excitation/emission wavelengths. Each experimental series included calibration wells of known MU or AMC concentrations and auto-fluorescence blanks. Laccase (1.10.3.2) activity was determined by a photometric assay based on the ABTS substrate (2,2'-Azino-bis-3-ethylbenzo-thiazolin-6-sulfonic acid). The experimental procedure was similar to that described above, and absorbance was measured at 420 nm (see more details in previous Chapters).

Statistical analyses

All variables were log or square root transformed when needed after testing for normality (Shapiro test) and homoscedasticity (Levene test). Soil variables and enzymatic activities were analysed by Generalized Linear Models (GLM) (p<0.05) with the factors fire regime, pine species and their interaction. The pine population (i.e., site) was nested within the factor fire regime. Separated analyses for each pine species were then conducted to check the fire regime effect.

The infrared spectral data matrices consisted of 75 samples and 4044 (NIR) or 2566 (MIR) columns, each corresponding to a wave frequency within the respective spectrum ranges (Figure 1). Both NIR and MIR spectral data were mathematically transformed by calculating the first derivate after standard normal transformation (Reeves *et al.*, 2002). Mean comparison tests (p<0.05) were performed on each spectral data set to identify the frequencies or the range of wave frequencies that significantly differed among fire regime and pine species. The effect of the pine species and the fire regime on edaphic variables, and NIR and MIR soil spectra were checked by multivariate analysis of variance (PERMANOVAs) and nonmetric multidimensional scaling (NMDS) with the function *adonis* and *metaMDS* in vegan R package, after calculating the Euclidean distance matrix of variables (Oksanen *et al.*, 2015). The dimensionality of the matrix of edaphic variables and of the NIR and MIR spectral matrices was reduced by principal component analyses (PCA) with the function *dudi.pca* in the ade4 R package. To check the impact of the factors fire regime and pine species on soils, first principal axes of these PCAs were separately analyzed by GLMs (p<0.05), and specific NIR and MIR spectral regions (Figure 1) were additionally analyzed: 1) Region A: the NIR and MIR overlapping region 5498-2198 cm^{-1} allowing the comparison of both techniques, and 2) Region B: 3036-2376 cm^{1} as a proxy of soil organic matter quality, since it is related to O-alkyl C (deriving primarily from cellulose and hemicelluloses but also from proteins and side chains from lignin), aromatic C (related to lignin) and carbonyl C (from aliphatic esters, carbonyl groups, and amide carbonyls) (Terhoeven-

Urselmans *et al.*, 2006; Ludwig *et al.*, 2008). All statistical analyses were performed using the software R 3.1.1 (R Core Team, 2014).

RESULTS

Forests of both pine species showed contrasted edaphic properties (Table 1; Figure 1). The pH, EC, OM, N and K were neatly higher in the case of *P. halepensis*, while the P content and the C/N rate were greater in *P. pinaster* soils (Table 1). In both cases, the high fire recurrence significantly increased the levels of N and P (Table 1). The high fire recurrence reduced the pH in *P. halepensis* soils, and the K content in *P. pinaster* soils. Either in *P. pinaster* or *P. halepensis* forests, the high fire recurrence decreased the C/N rate of soils (Table 1).

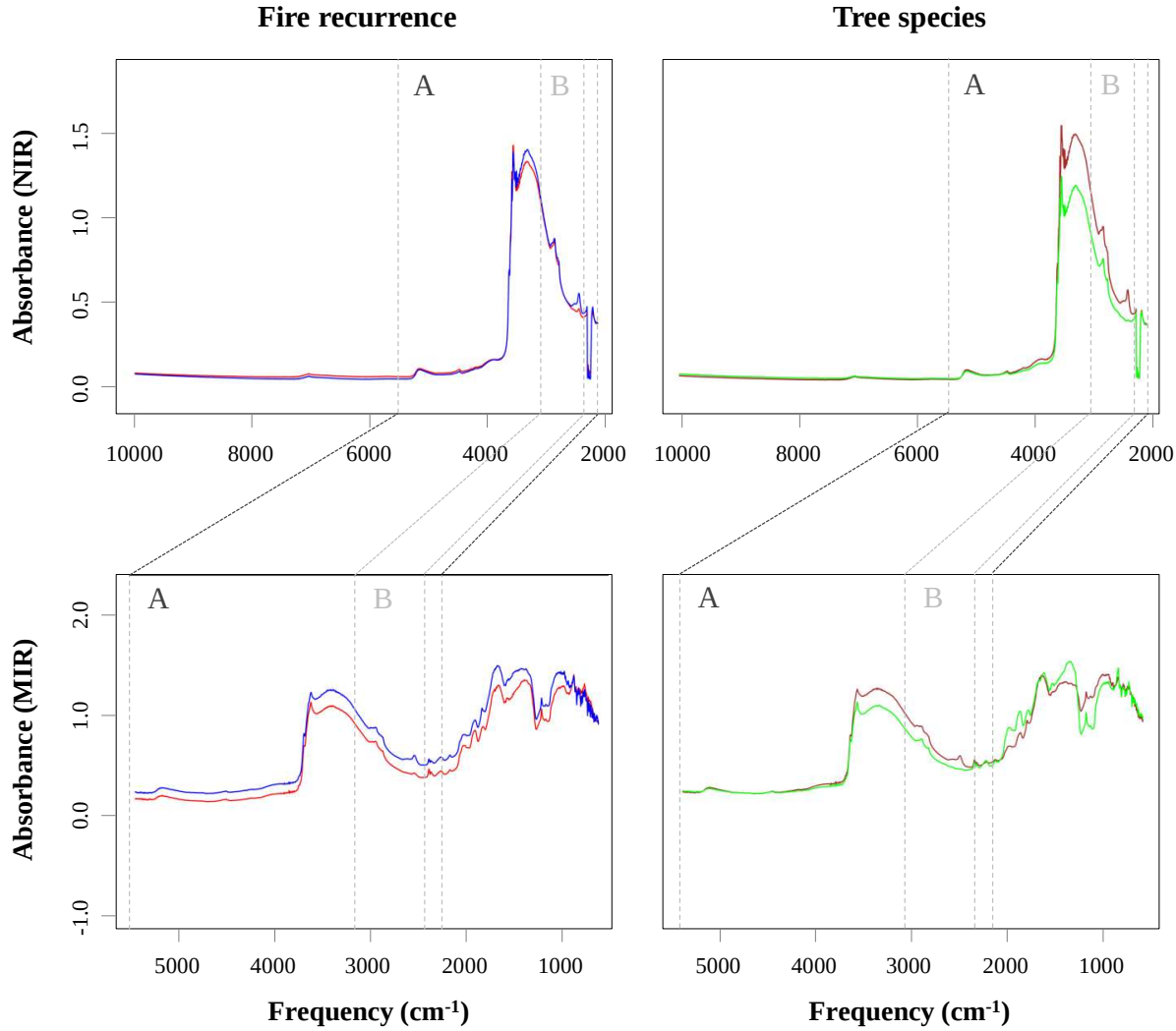

Figure 1 | Near and mid infrared spectral data (NIR and MIR respectively) by fire regime (blue = Low and red = High fire recurrence), and tree species (green = *Pinus pinaster*; brown = *Pinus halepensis*).

Table 1 | (a) Soil properties analyzed by General Linear Model (GLM) testing the effect of fire regime, pine species and their interaction. F values and significance level: *p<0.05; **p<0.01;***p<0.001. Significant differences are highlighted in bold. EC = electric conductivity, OM = organic matter, N = nitrogen, P = phosphorous, K = potassium, C/N = carbon/nitrogen ratio. (b) Effect of fire regime separately analyzed by pine species *Pinus pinaster* and *Pinus halepensis*. Values = means ± SE. For each pine species, different letters denote significant differences between low (LoFi) and high (HiFi) fire recurrence.

		pH	EC (µS/cm)	OM (%)	N (%)	P (mg/kg)	K (mg/kg)	C/N
(a) Main test	Fire	0.63	1.08	1.30	9.19**	5.58*	0.22	26.27***
	Species	101.73***	181.95***	24.82***	72.58***	20.81***	81.50***	129.99***
	Fire x species	7.11**	20.92***	17.53***	19.68***	2.56	12.40***	0.07
(b) Fire effect by pine species								
P. pinaster	LoFi	5.9 ±0.2 a	146.1± 19.5 a	7.6± 1.4 a	**0.12 ± 0.02 a**	**3.3 ± 0.5 a**	**102.5 ± 12.6 b**	**38.4 ± 1.1 b**
	HiFi	5.9 ± 0.1 a	139.9 ± 16.0 a	7.4± 0.7 a	**0.13 ± 0.01 b**	**4.1 ± 0.1 b**	**74.6 ± 4.3 a**	**33.6 ± 1.3 a**
P. halepensis	LoFi	**7.6 ± 0.1 b**	565.1 ± 71.1 a	12.9± 1.9 a	**0.26 ± 0.04 a**	**2.1 ±0.3 a**	179.7 ± 11.5 a	**26.6 ± 1.1 b**
	HiFi	**7.2 ± 0.1 a**	541.7 ± 34.7 a	14.5 ±1.7 a	**0.38 ± 0.04 b**	**2.8 ± 0.2 b**	210.7 ± 23.4 a	**22.5 ± 0.7 a**

The contrasted edaphic environment determined clear functional divergences in *P. pinaster* and *P. halepensis* forests (Figure 2). Generally, higher activities of C degrading enzymes were found in *P. pinaster* soils (i.e., glucosidase, cellobiohydrolase, xylosidase), while enzymes that mobilize N and P (i.e., chitinase and phosphatase) were more active in *P. halepensis* soils (Figure 2). The fire regime differently affected soil enzymatic activities depending on the pine species (Figure 2). In recurrently burned soils, laccase, chitinase and phosphatase activities increased in *P. halepensis* forests, while in *P. pinaster* the glucuronidase activity decreased (Figure 2).

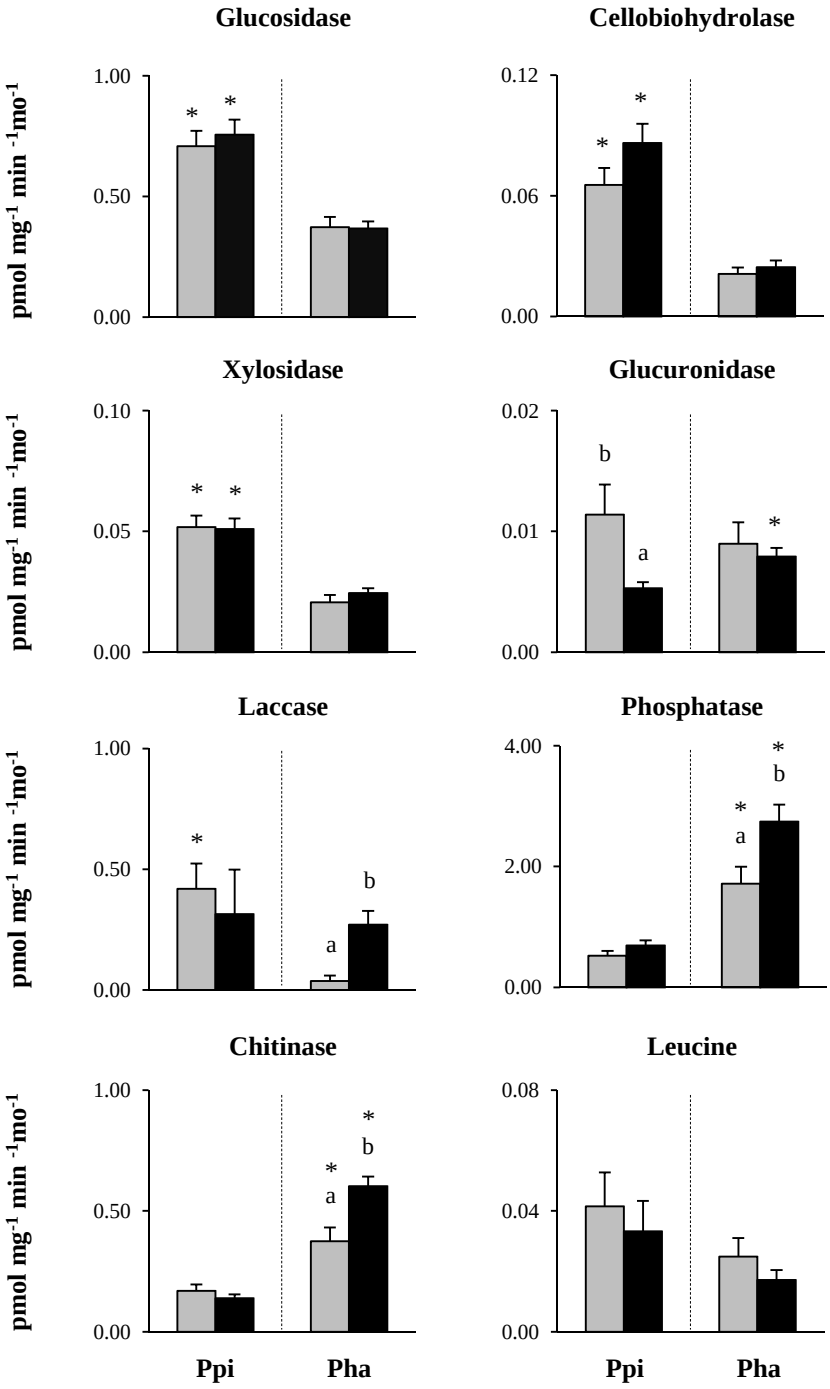

Figure 2 | Effect of the fire regime in the enzymatic activities (means ± SE) of *Pinus pinaster* (Ppi) and *Pinus halepensis* (Pha) forest soils. For each pine, different letters denote significant differences between low (LoFi, grey bars) and high (HiFi, black bars) fire recurrence, while for each fire treatment, asterisks denote significant differences between pine species ($p<0.05$).

Both factors, the fire regime and the pine species, greatly affected the near and mid-infrared soil spectra (Figure 1; Figure S2). Significant differences between P. pinaster and P. halepensis soils were detected for the38 % of NIR and the 29% of MIR bands in the total respective spectra. Independently of the tree species, the 27% of NIR and the 21% of MIR bands were different in soils under high and low fire recurrence. When separately analyzed by pine species, the effect of the fire regime was pronounced in P. halepensis forests, with the 24% and the 22% of NIR and MIR bands significantly different between low and high fire recurrence (Figure S2). In the case of P. pinaster, the 17 % and the 13 % of NIR and MIR bands were different between LoFi and HiFi (Figure S2). These differences were accentuated in the overlapping Region A, and in the Region B, for both NIR and MIR approaches (Figure 1; Figure S2).

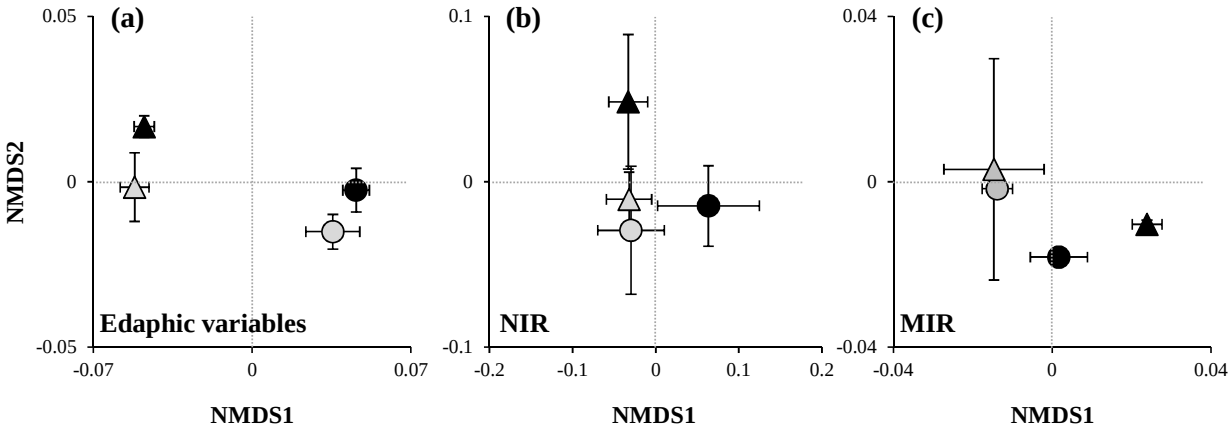

Figure 3 | Nonmetric multidimensional scaling (NMDS) of (a) Edaphic variables (see Table 1) (k=2; stress=0.09; R^2=0.99), (b) NIR near (k=2; stress=0.13; R^2=0.98) and (c) MIR mid (k=2; stress=0.05; R^2=0.99) infrared spectral data, for the factors fire regime (grey=Low and black=High fire recurrence), and pine species (triangle = *Pinus pinaster* and circle = *Pinus halepensis*).

Ordination and PERMANOVA analyses showed that the edaphic variables clearly separated by pine species, but it did not recover the fire regime effect (Figure 3a; Table S1). However, a marked fire regime influence on the quality of soils was revealed by the infrared spectra, particularly significant in the case of MIR (Figure 3; Table S1). In fact, when modeling the principal components of NIR and MIR soil spectra, the effect of fire recurrence was significantly retrieved by one among the three first principal axes for both pine species (Table 2). When analyzed by specific regions (Table 2; Figure 4) (i.e., overlapping Region A, and the Region B proxy of organic matter quality), the fire regime effect was more pronounced in the case of *P. halepensis* than *P. pinaster* (Table 2; Figure 4).

Table 2 | Effects of the fire regime on the (a) near NIR and (b) mid MIR soil infrared spectra by pine species (Ppi = *P. pinaster*; Pha = *P. halepensis*), analysed by General Linear Model (GLM) ($p<0.05$). Principal components (PCA analysis) of total and partial regions of the spectra were considered as response variables; percentages of variance explained are shown in brackets. F values; ns=not significant; .p<0.08; *p>0.05; **p<0.01;***p<0.001; significant values in bold.

	(a) Near Infrared NIR				**(b) Mid Infrared MIR**			
				Total (9997-2198 cm^{-1})				
	% Variance	***P. pinaster***	*% Variance*	***P. halepensis***	*% Variance*	***P. pinaster***	*% Variance*	***P. halepensis***
PC1	*(19.7%)*	1.06ns	*(23.8%)*	**5.69***	*(29.3%)*	**3.23.**	*(32.5%)*	1.73ns
PC2	*(11.0%)*	0.31ns	*(10.4%)*	0.16ns	*(24.1%)*	2.38ns	*(17.3%)*	**8.69****
PC3	*(9.6%)*	**10.66****	*(8.8%)*	0.59ns	*(19.3%)*	**12.35****	*(13.9%)*	2.87ns
Total	*40.3%*		*43.0%*		*72.7%*		*63.7%*	
				Region A (5498-2198 cm^{-1})				
	% Variance	***P. pinaster***	*% Variance*	***P. halepensis***	*% Variance*	***P. pinaster***	*% Variance*	***P. halepensis***
PC1	*(25.3%)*	1.25ns	*(27.3%)*	**11.78****	*(30.6%)*	0.51ns	*(34.3%)*	**3.26.**
PC2	*(14.6%)*	**3.69.**	*(17.7%)*	**3.89.**	*(27.2%)*	**4.41***	*(18.0%)*	**6.37***
PC3	*(10.4%)*	0.02ns	*(11.3%)*	**19.52*****	*(20.8%)*	0.15ns	*(14.2%)*	**5.35***
Total	*50.4%*		*56.4%*		*78.6%*		*66.5%*	
				Region B (3036-2376 cm^{-1})				
	% Variance	***P. pinaster***	*% Variance*	***P. halepensis***	*% Variance*	***P. pinaster***	*% Variance*	***P. halepensis***
PC1	*(38.9%)*	0.04ns	*(51.5%)*	**11.67****	*(25.2%)*	0.03ns	*(32.3%)*	**22.13*****
PC2	*(28.9%)*	0.04ns	*(17.4%)*	0.01ns	*(21.5%)*	0.96ns	*(23.1%)*	**16.11*****
PC3	*(13.2%)*	**12.67****	*(10.8%)*	**11.62****	*(16.4%)*	0.49ns	*(12.1%)*	2.41ns
Total	*80.9%*		*79.8%*		*63.0%*		*67.5%*	

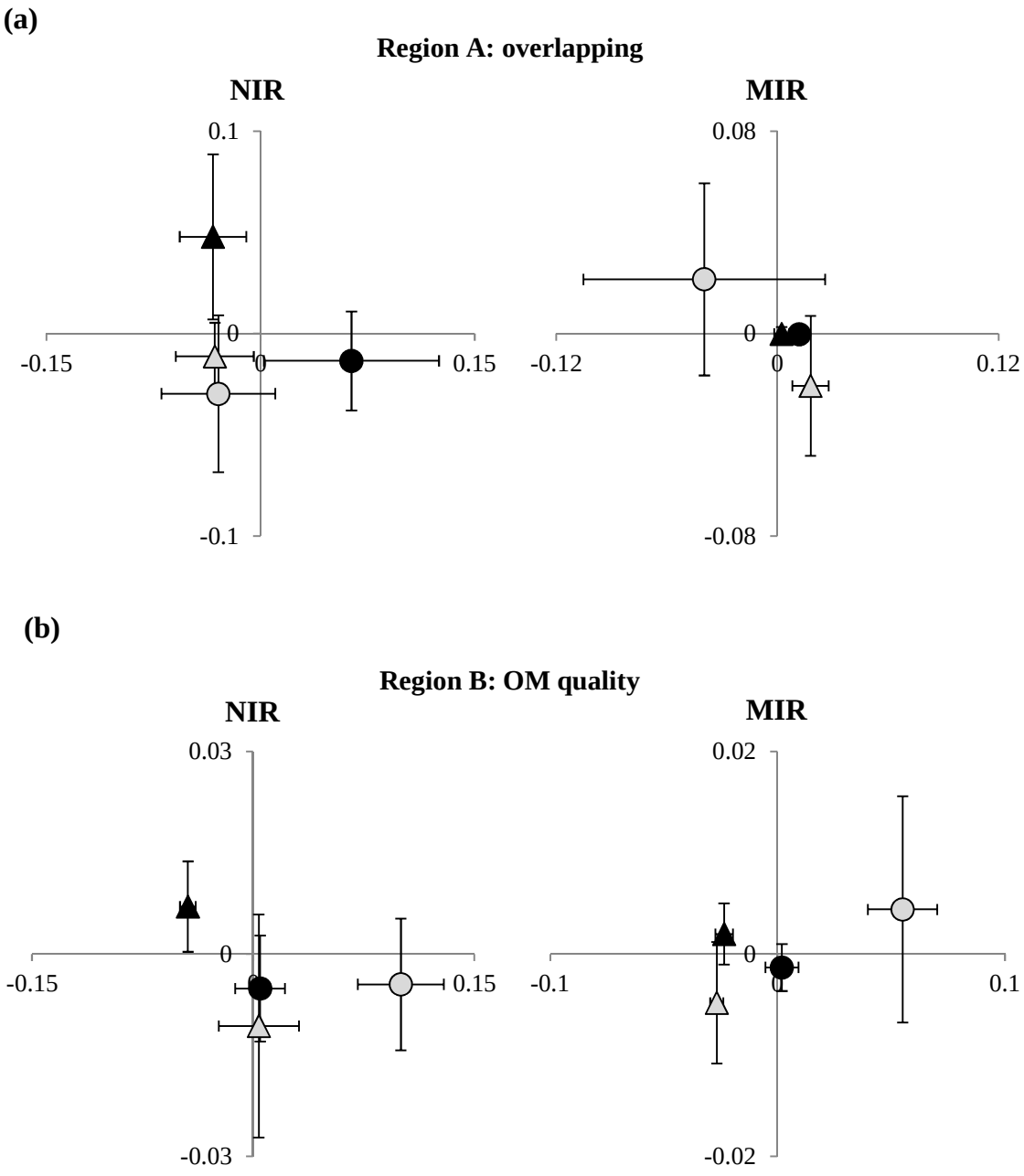

Figure 4 | Nonmetric multidimensional scaling (NMDS) of (a) Region A (NIR k=2; stress=0.13; R^2=0.98; MIR k=2; stress=0.06; R^2=0.99), and (b) Region B (NIR k=2; stress=0.04; R^2=0.99; MIR k=2; stress=0.04; R^2=0.99) of near NIR and mid MIR infrared spectral data, for the factors fire regime (grey=Low and black=High fire recurrence), and pine species (triangle = *Pinus pinaster*; circle = *Pinus halepensis*).

DISCUSSION

Our results reveal the fire regime as a key ecological factor determining the global quality of soils in Mediterranean forests, despite the contrasted soil environment of *P. pinaster* and *P. halepensis* populations. Although both are fire-prone forests, different functional responses to fire recurrence are observed depending on the tree species. The NIR-MIR spectral analyses are demonstrated to be good integrative proxies of soil quality catching imprint effects of the fire regime, which is a very important ecological factor in the Mediterranean area.

Contrasted soil environment in forests of two Mediterranean pine species

Forests of the two pine species studied showed contrasted physical-chemical soil properties, with higher values of most variables in *P. halepensis* soils compared with *P. pinaster* soils, except for the content of phosphorous and the C/N rate. The primary production of Mediterranean forests is highly constrained by the availability of water and hence, soils usually present low organic matter contents (Rodà *et al.*, 1999). In parallel, these soils generally show nutrient deficiencies, especially P in calcareous soils (Mayor and Roda, 1994; Sardans *et al.*, 2004), although it is well established that calcareous bedrocks (majority in *P. halepensis*) are usually nutrient-richer than siliceous ones (majority in *P. pinaster*) (Ojeda *et al.*, 2010). On the other hand, the distinct C:N rate might be related to a differential recalcitrance of SOM between both kinds of pine forests (Hedo *et al.*, 2015). The NIR-MIR techniques used in this study pointed out to this possibility, giving an integrated picture of these differences that were particularly evident in the spectral overlapping region and that focusing on the organic matter quality with clear differences between forests of both pine species. Previously, Cécillon *et al*. (2009) proposed these techniques as good proxies of "global" quality of soils, and recently Akroume *et al*. (2016) confirmed these assertions emphasizing their use without any preliminary calibration, as previously discussed (Chapter 1).

The litter quantity and quality determines the formation and composition of SOM (Schweitzer *et al.*, 2004; Wang *et al.*, 2010; Stewart *et al.*, 2011; Wardle *et al.*, 2012), where microbial communities have a key role since SOM decomposition is primarily driven by fungi and bacteria (McGuire and Treseder, 2010). Moreover, there is increasing evidence that microbial residues play also a significant role as SOM precursors (Clemmensen *et al.*, 2013; Cotrufo *et al.*, 2013; Fernandez *et al.*, 2016). In Mediterranean conditions characterized by periods of drought, probably fungi overcome bacterial communities even as soil residuals (Curiel Yuste *et al.*, 2011; Flores-Rentería *et al.*, 2015), whose mycelium is often composed by recalcitrant substances such as melanin (Treseder and Lennon, 2015). In our study, intrinsic differences due to the bedrock type (local conditions closed linked to the pine species distribution) and thus differences in litter

quality together with soil microbial residuals would have influenced the SOM formation, as suggested for other Mediterranean forests (Garcia-Pausas *et al.*, 2004). These differences could have been decisive for microbial communities and so for the ecosystem functioning (Strickland *et al.*, 2009; Burke *et al.*, 2011). In fact, a clear dissimilar functionality was observed in soils of each pine species. In general, higher activities of enzymes related to carbon degradation were found in *P. pinaster* soils (with greater C:N ratios), while higher activities of enzymes mobilizing nitrogen and phosphorous were found in *P. halepensis* forest soils, indicating a possible dependence of substrate availability (Hernández and Hobbie, 2010), although in the case of P other mechanisms could operate. As Sinsabaugh *et al.* (2008) stated, soil pH is a key agent determining the availability of nutrients and SOM composition, and thus influencing the enzymatic activity of soil. Moreover, the pH has been described as a major driver structuring soil fungal and bacterial communities (Fierer and Jackson, 2006; Rousk *et al.*, 2010; Stéphane Uroz *et al.*, 2013; Coince *et al.*, 2014; Tedersoo *et al.*, 2014; Rincón *et al.*, 2015). Because functional responses of fungi to resource availability have been demonstrated (Courty *et al.*, 2016), the different soil enzymatic activity of both pine forests might also indicate shifts in the fungal demand of carbon, nitrogen and/or phosphorus (Sinsabaugh and Moorhead, 1994).

Impact of the fire regime in Mediterranean pine forest soils

Either in *P. pinaster* or *P. halepensis* forests, the high fire recurrence decreased the C/N rate in soils and increased the contents of nitrogen and phosphorous. Furthermore according to our hypothesis, the fire regime determined the overall quality of soils. The quantity of SOM did not vary with the fire recurrence, while its global quality did. In the short-term, forest wildfires highly modify the physical, chemical and biological soil properties, causing significant negative effects as removal of organic matter, deterioration of both structure and porosity, loss of nutrients, erosion and/or alteration of microbial composition (Ballard, 2000; González-Pérez *et al.*, 2004; Certini, 2005). However, soil seems to recover in the long-term (Smithwick *et al.*, 2009; Dunnette *et al.*, 2014; Hedo *et al.*, 2015). In particular, Smithwick et al. (2009) showed that total ecosystem N was rapidly recovered following stand replacing fires aided by the fast regeneration of *Pinus contorta*, a fire-prone species producing serotinous cones, as it might have happened for *P. pinaster* and *P. halepensis* in our study. Baldock et al. (1997) showed that SOM recovery in burned areas depended on both the quantity and quality of litter inputs and the rate of decomposition by the remaining soil microbiota. In fact, recurrent fires can change the recalcitrance of organic matter compounds by defining the plant community composition (i.e., the chemical quality of plant inputs) (Guénon *et al.*, 2011), and this different SOM recalcitrance can further control post-fire N mineralization (Martí-Roura *et al.*, 2014). Accordingly with this, Hart

et al. (2005) established that where recurrent fire had shaped the evolutionary history of a forest, the indirect effects on soil are primarily mediated by fire-induced changes in the plant community and into a lesser extent on direct fire effects on soil. Therefore in consonance with these previous studies, our results may suggest that, in the long term, indirect effects of recurrent fires such as changes in the plant and/or the microbial communities as well as on the productivity of recurrently burned vegetation, would determine the SOM quality and relevant ecosystem processes.

When separately analyzed by pine species, the effect of the fire regime was highly pronounced in *P. halepensis* forests, especially in the Region B (i.e., proxy of SOM quality). This was further reflected on the ecosystem functioning, since extracellular enzymatic activities in *P. pinaster* soils barely responded to the fire regime, while those related to N and P mobilization (i.e., chitinase and phosphatase) and the degradation of recalcitrant C compounds (i.e., laccase) increased in *P. halepensis* populations subjected to high fire recurrence. Direct fire effects on SOM quality and/or microbial communities, together with inherent local conditions, could have determined the enzymatic activity of these soils (Carreiro *et al.*, 2000). According to this, Henry (2012) established that the disturbance caused by fire can alter the succession of plant communities indirectly affecting soil functioning in the long term. Across a fire chronosequence, Holden et al. (2013) observed that changes in extracellular enzyme activities were associated with changes in total fungal hyphal length abundance. Our results might also reflect a possible predominance of certain microorganisms with similar functional traits favoured under high fire recurrence in *P. halepensis* forests, as previously reported (Rincón *et al.*, 2014; Glassman *et al.*, 2016; Pérez-Valera *et al.*, 2016). Moreover, although the two pine species show high resistance to fire (Keeley *et al.*, 2011), our results could indicate a dissimilar resilience to a high fire recurrence. Overall our study confirms that the fire regime is an important ecological factor shaping the quality and functioning of Mediterranean forest soils. Better understanding the fire ecology of Mediterranean forests, especially concerning the belowground soil environment is necessary to promote a sustainable management of these ecosystems.

SUPPORTING INFORMATION

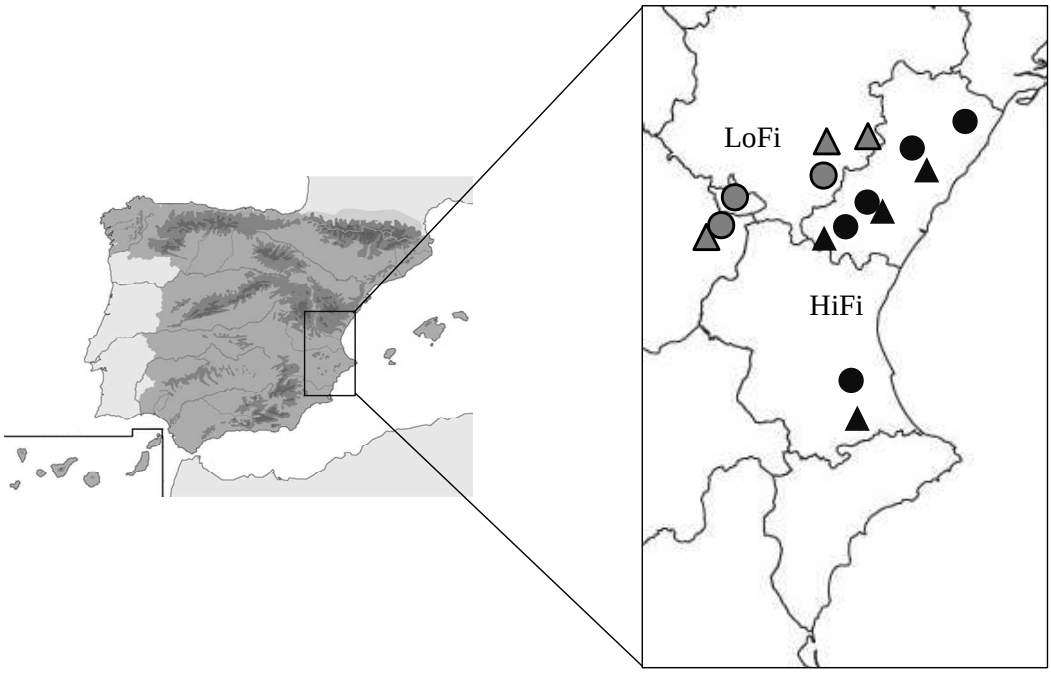

Figure S1 | Location of the fifteen pine populations of *Pinus pinaster* (triangles) and *Pinus halepensis* (circles), growing in areas of low (LoFi, grey) and high (HiFi, black) fire recurrence. See additional details in Chapters 6-7.

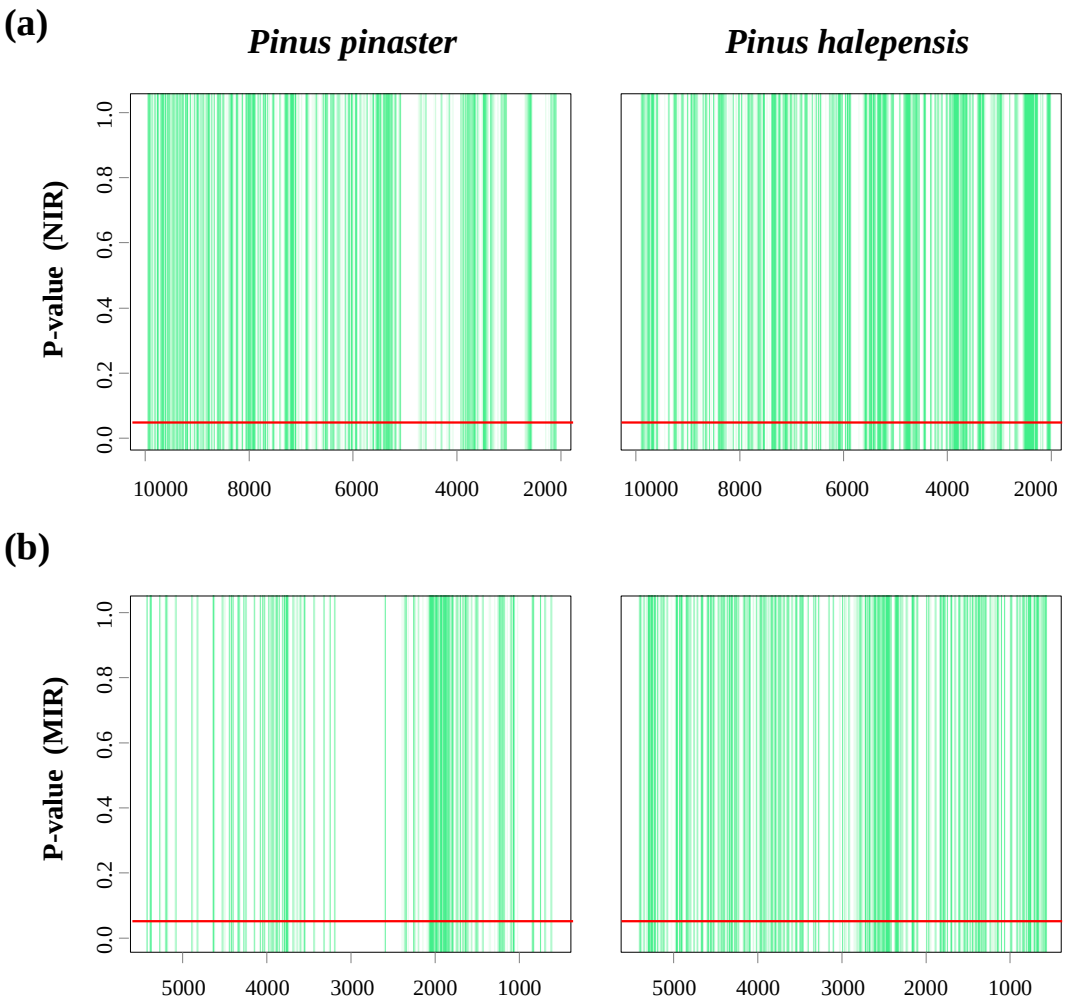

Figure S2 | Mean comparison test on (a) near NIR and (b) mid MIR infrared spectral data to identify the frequencies that significantly differ with the fire regime for each pine species. Green bars denote significant differences between low and high fire recurrence ($p > 0.05$).

Table S1 | Effect of the fire regime, the pine species and their interaction on the edaphic variables and the NIR-MIR soil spectral data analysed by permutation variance analyses (PERMANOVA). R^2, F-ratio and p value: ns = not significant, .p<0.08, *p<0.05; **p<0.01;***p<0.001). Region A: overlapping region of the spectra; Region B: proxy of organic matter quality.

	Edaphic variables		**NIR**			**MIR**	
	F	**R^2**		**F**	**R^2**	**F**	**R^2**
			Total Spectrum (9997-2198 cm^{-1})				
Fire	0.08ns	0.00	*Fire*	**2.17.**	**0.03**	**2.99***	**0.04**
Species	**98.45*****	**0.58**	*Species*	**6.40*****	**0.08**	1.44ns	0.02
Fire x Sp	0.50ns	0.00	Fire x Sp	1.77ns	0.02	0.95ns	0.01
			Region A (5498-2198 cm^{-1})				
			Fire	**2.17.**	**0.03**	**2.13*****	**0.04**
			Species	**6.40*****	**0.08**	**3.52*****	**0.04**
			Fire x Sp	1.77	0.02	**2.48*****	**0.03**
			Region B (3036-2376 cm^{-1})				
			Fire	**4.73***	**0.05**	**4.93****	**0.05**
			Species	**19.50*****	**0.19**	**17.26*****	**0.18**
			Fire x Sp	**5.8****	**0.06**	**5.84****	**0.06**

Chapter 6

Feedbacks of host tree and root-tip ectomycorrhizal communities in Mediterranean pine forests under distinct fire regime

INTRODUCTION

The evolutionary history of Mediterranean ecosystems is tightly linked to fire (Keeley *et al.*, 2011). Recurrent burning has markedly structured plant communities in this area (Verdú and Pausas, 2007), where many species have developed evolutionary mechanisms of resistance to fire (Tapias *et al.*, 2004; Pausas, 2015). A good example of fire-adaptive trait is serotiny, i.e., the retention of matured seeds in closed cones for more than a year, evolved by some representative Mediterranean pine species (Hernández-Serrano *et al.*, 2013; Budde *et al.*, 2014). Together with aboveground impacts, recurrent forest wildfires affect belowground diversity, as well as mechanisms crucial for the fitness and survival of trees such as mutualistic and antagonistic interactions, with functional costs that still remain largely unknown (Johnson *et al.*, 2012; Pausas, 2015).

Mutualistic ectomycorrhizal (ECM) fungi are key players in forests because they improve the uptake and transfer of water and nutrients to trees (Smith and Read, 2008). Besides, these fungi channel the photosinthetically fixed carbon into the belowground, influencing soil carbon storage and nutrient cycling (Talbot *et al.*, 2008; Clemmensen *et al.*, 2013). This symbiosis can provide up to the 80% of nutrients to trees and alleviate their hydric stress (Kivlin *et al.*, 2013; van der Heijden *et al.*, 2015), and can be especially relevant in severe environments e.g., recurrent fire and drought conditions in Mediterranean ecosystems (Egerton-Warburton *et al.*, 2007; Querejeta *et al.*, 2007; Prieto *et al.*, 2016). On the fungal side, ECM communities are affected by fire mainly through vegetation damages and altered soil properties (Hart *et al.*, 2005; Rincón and Pueyo, 2010; Buscardo *et al.*, 2015), conditions that may favor fire-prone fungi (Rincón et al. 2014; Glassman et al. 2016). In fact, in closed-cone pine forest populations with historical highly recurrent and intense fires, certain fungi are selected remaining long term in the soil spore banks (Baar *et al.*, 1999; Glassman *et al.*, 2016).

In our study, we targeted two representative Mediterranean tree species, *Pinus pinaster* Ait. and *Pinus halepen*sis Mill., amply distributed in the Iberian Peninsula. While the Maritime pine (*P. pinater*) usually grows in acid soils at 700-1700 m altitude, the Aleppo pine (P. *halepen*sis) normally grows in basic substrate and below 800 m (Ruiz *et al.*, 2009). *P. halepensis* typically distributes in warm and dry areas, even under extreme drought induced either by climate or by constituents (e.g., marls, gypsum, rocky slopes) (Ruiz *et al.*, 2009). Both species have a wide range of life histories related to fire adaptation, although they show variable strategies for cone bearing and seed dispersal (Tapias *et al.*, 2004). These pines display great colonizing abilities (Barbéro *et al.*, 1998) and show fire-adaptive traits, e.g., serotiny (Pausas, 2015). Beyond the tree species identity, even different tree genotypes and phenotype variants can directly influence their associated ECM fungal communities, which are tightly linked to the host through

specialized symbiotic structures (Gehring and Whitham 1991; van der Heijden *et al*. 2015; see Chapters 2-4). Additionally, the natural regeneration and dynamics of these pine forests critically relays on ECM fungal communities, because pine species are obligatory mycorrhizal (Smith and Read, 2008; Nuñez *et al.*, 2009).

Based on these premises, we expected that i) the ECM fungal communities associated with root tips of close-cone (i.e., serotinous) pine populations in areas of high fire recurrence would diverge from not serotinous populations settled in low fire recurrence areas. Despite the low specificity of the mycorrhizal symbiosis, we also expected that ii) due to the contrasted ecology of these pine species, *P. pinaster* and *P. halepensis* would harbor dissimilar root-tip ECM fungal communities. Moreover, given that ECM fungi are crucial for soil carbon turnover and nutrient cycling (Lindahl and Tunlid, 2015), iii) fire-induced shifts in the ECM communities were expected to affect functions linked with these relevant ecosystem processes.

In order to address these predictions, we characterized the structure of the root-tip ECM fungal community of *P. pinaster* and *P. halepensis* in natural populations with different serotiny degree, and settled in areas with contrasted fire regime (Hernández-Serrano *et al.*, 2013). Additionally, fungal enzymatic traits, related with carbon turnover and nutrient's mobilization, were determined on excised ECM root-tips. These are processes directly implicated in the exchange of resources that support most mycorrhizal symbioses, and many essential ecosystem functions (Johnson et al. 2012).

MATERIAL AND METHODS

Study sites and sampling

The study was conducted in natural populations of two Mediterranean pine species in eastern Spain. Seven populations of *P. pinaster* (Ppi) and eight of *P. halepensis* (Pha) were selected in sites with different fire regimes: low (LoFi) and high (HiFi) fire recurrence (Table S1). In the area, fire is tightly linked to Mediterranean climatic conditions (i.e., to drought) (Pausas *et al.*, 2004), and fire history evidences a much shorter fire return interval in HiFi areas compared with LoFi areas (Abdel Malak and Pausas, 2006). Nine populations are located in sites where crown-fires are historically frequent and most regeneration events are driven by fire (HiFi populations), while the other six populations are located in areas where most regeneration events are independent of fire because fire events are rare (LoFi populations) (see Figure S1 in Chapter 5) (Pausas *et al.*, 2004; Hernández-Serrano *et al.*, 2013). From a microevolutionary point of view, this distinct fire regime has induced a sharp serotiny divergence within these populations (Hernández-Serrano *et al.*, 2013).

In spring 2013, five trees per population separated of more than 10 m were selected in a total of 15 populations (n = 75). Under each tree, the litter was removed 1 m far from the trunk and samples were obtained by excavating 10 x 10 x 20 cm soil holes at the four orientations. These four samples per tree were pooled into a combined sample and kept at 4 ºC in plastic bags until processing. Once in the lab, roots were separated from bulk soil, coarse ones discarded (diameter > 2 mm), and remaining roots gently washed with tap water over 2 and 0.5 mm sieves for collecting short roots. All ectomycorrhizal (ECM) root tips were carefully selected and sorted per sample under a stereomicroscope for further enzymatic and molecular analyses. Remaining bulk soil was air dried and sieved (2 mm) for chemical analyses as previously described in Chapter 5.

Enzymatic tests

The fungal community functioning was evaluated on excised ECM root tips by measuring activities of eight hydrolytic and oxidative exoenzymes secreted by fungi. Seven enzymatic tests were based on fluorogenic substrate release, methylumbelliferone (MU) e.g. β-glucosidase (EC 3.2.1.3) and cellobiohydrolase (EC 3.2.1.91) that degrade cellulose, xylosidase (EC 3.2.1.37) and β-glucuronidase (EC 3.2.1.31) implied in the degradation of hemicellulose, phosphatase acid (EC 3.1.3.2) involved in the mobilization of phosphorous, chitinase (EC 3.2.1.14) which hydrolyses chitin, or methylcoumarine (AMC) for L-leucineaminopeptidase (3.4.11.1) related to the mobilization of N from peptidic substrates. The Lacasse (1.10.3.2) activity was determined by a photometric assay based on ABTS substrate (2,2'-Azino-bis (3-ethylbenzo-thiazolin-6-sulfonic acid). This enzyme is related to the degradation of recalcitrant compounds such as lignin. Enzymatic activities were determined following the protocol described by Courty *et al*. (2005), with modifications. A total of 280 ECM-tips were randomly collected per sample and separated in subsets of 7 ECM-tips with 5 replicates per each enzymatic test, adjusting buffers and substrates proportionally to that described by Courty *et al*. (2005) for a single root tip. For each replicate, seven ECM-tips were pooled in an Eppendorf tube and incubated in 400 µl of buffer and 200 µl of substrates during the corresponding time for each enzyme (Courty *et al.*, 2005), after which 100 µl of the respective enzymatic reaction mix was added to 100 µl of stopping buffer in 96-well microplates. Enzymatic activities were measured in a Victor microplate reader (Perkin-Elmer Life Sciences, Massachusetts, USA), with 355/460 nm excitation/emission wavelengths for the fluorogenic assays and 415 nm for laccase. After reading, the ECM-tips of each replicated pool were scanned and their area calculated with the software ImageJ 1.49. Enzymatic activities were expressed in pmol $min^{-1}mm^{-2}$.

Molecular analyses

Per each measured enzyme, the ECM-tips were pooled (n=35), added of a pinch of polyvinylpolypyrrolidone (PVPP), and the DNA extracted with the Invisorb®DNA Plant HTS 96 Kit/C kit (Invitek GmbH, Berlin, Germany), making a total of 600 DNA extractions (75 samples x 8 enzymes) corresponding to 280 root tips per tree.

The internal transcribed spacer region ITS-1 of the nuclear ribosomal DNA was amplified with the primer pair ITS1F-ITS2 (Gardes and Bruns, 1993) adapted for Illumina-MiSeq. PCR amplifications (3 min 94 ºC, 30 cycles of 1 min 94 ºC, 30 s 53 ºC and 45 s 72 ºC, with a final step of 10 min 72 ºC) were conducted in a Verity Thermal Cycler (Life Technologies), and each sample was amplified in three independent 20 µl reactions, each containing 2 µl of 10x polymerase buffer, 2.4 µl of 25 mM $MgCl_2$, 1.12 µl of 10 mg ml^{-1} BSA, 0.4 µl of 10 mM Nucleotide Mix, 0.4 µl of 10 mM forward/reverse primers and 0.2 µl of AmpliTaqGold polymerase (5 U ml^{-1}) (Applied Biosystems, Carlsbad, CA, USA). Negative controls without DNA were included in all runs to detect possible contaminations. Independent reactions were combined per sample, and each PCR product was purified (UltraClean PCR clean-up kit of MoBio, Carlsbad, CA, USA), quantified (PicoGreen, Life Technologies, Carlsbad, CA, USA), and finally pooled in an equimolar library containing 75 samples. Sequencing was carried out on an Illumina MiSeq sequencer in an external service (Parque Científico de Madrid, Spain).

Bioinformatic analyses

Sequences were de-multiplexed according to their tags, filtered and trimmed using the *fastq_filter* command and *fastq_truncqual* option of Usearchv.7.0.1001 (Edgar, 2013) for eliminating quality scores ≤ 10. FLASH was used to merge reads at 97 % of similarity using 110 and 160 of minimum and maximum overlap respectively, and the 58.3 % of sequences was retained (4205677 out of the initial set of 7215915 sequences). Sequences were dereplicated with the *derep_fulllength* Usearch command. De-replicated sequences were then sorted by decreasing abundance, and singletons discarded with the *sortbysize* Usearch command. Finally, 4116377 sequences were retained. Molecular operational taxonomic units (MOTUs) were generated from abundance-sorted sequences using the *cluster_otus* Usearch command with a 97 % similarity threshold. Extracted ITS sequences were then mapped against the MOTU representative sequences using the *usearch_global* Usearch command. Taxonomic assignation of representative sequences for each MOTU was done by using the Basic Local Alignment Search Tool (BLAST) algorithm v 2.2.23 (Altschul *et al.*, 1990) against the UNITE database (Kõljalg *et al.*, 2013). Fungal taxonomic assignment served to identify those MOTUs closely related to recognized ECM taxa (Tedersoo and Smith, 2013; Tedersoo *et al.*, 2014; Nguyen *et al.*, 2016). The 86 % of the

inferred sequences corresponded to ECM fungi and these were used for all subsequent analyses. Data were deposited in the Sequence Read Archive (http://www.ncbi.nlm.nih.gov/sra) as PRJNA324224.

Statistical analyses

Shapiro and Levene tests were performed to test the normality and homocedasticity of variables that were log or square root transformed when needed. The fungal community functioning (eight enzymatic activities), and the alpha-diversity (i.e., number of MOTUs and the number of reads as covariate) of total and representative fungal guilds (i.e., phyla and families) were first modeled by General Linear Models (GLM) with the factors fire regime, tree species and their interaction. The site (i.e., pine population) was nested within the factor fire regime. Separated models by tree species were also run with fire regime as fix factor and the site nested (n = 35 for *P. pinaster* and n = 40 for *P. halepensis*).

To identify representative fungal MOTUs of each fire regime per pine species, the Indicator Species Analysis (with MOTUs >100 reads) was carried out (p<0.05) with the function *multipatt* in the indicspecies R package (Cáceres *et al.*, 2013).

Bray-Curtis distance matrices of fungal species were calculated based on the abundance matrix of MOTUs previously normalized (i.e. variance stabilization) according to McMurdie and Holmes (2014) by using the DESeq R package (Anders and Huber, 2012). Over this matrix, the beta-diversity of total and by phyla fungal communities was calculated with the functions *betadisper* and *permutest* in the vegan R package (Anderson *et al.*, 2006; Oksanen *et al.*, 2015), considering the factors fire regime, tree species and their interaction.

Fungal community assemblage was analyzed by multivariate analysis of variance (PERMANOVAs) with the function *adonis* and by nonmetric multidimensional scaling (NMDS) with the functions *metaMDS* and *isoMDS* in vegan and MASS R packages using normalized abundance and presence/absence matrices (Oksanen *et al.*, 2015). The *envfit* function in the vegan R package was used to fit the enzymatic activities, edaphic variables and tree variables (serotiny=number of closed cones; DBH=diameter at breast height as proxy of productivity) into the NMDS space checking for significant correlations with ECM fungal communities.

All statistical analyses were carried out with the R software v3.0.2 (R Core Team, 2014).

RESULTS

Enzymatic activities of ECM root tips

The enzymatic activity of the ECM root tips was dependent on both, the pine species and the fire regime (Figure 1; Table S2). In LoFi populations, the enzymatic activity of ECM root tips was very similar for both pines, except in the case of cellobiohydrolase and phosphatase that were respectively higher and lower for *P. halepensis* compared with *P. pinaster* (Figure 1). By contrast, in HiFi populations, the activity of most of the C-degrading enzymes (i.e., cellobiohydrolase, xylosidase and glucuronidase) and of those mobilizing N (i.e., chitinase and leucine) was higher for *P. halepensis* than *P. pinaster*, with the exception of laccase (Figure 1).

Concerning the fire regime effect, the high fire recurrence reduced the hemicellulose degrading activity (i.e., xylosidase and glucuronidase) of *P. pinaster* root-tips and that of enzymes mobilizing N and P (Figure 1). For both pine species, the high fire recurrence significantly increased the laccase activity (Figure 1).

Sequencing yields and taxonomic identification of fungal community

A total of 501 ECM fungal MOTUs corresponding to 3182548 sequences were identified (Table S3). The 47.1% of MOTUs were shared by the two pine species, and ~26% of them exclusively found forming ECM with one of them (Figure 2a). The 54.8% of MOTUs were found at both fire regimes, while ~21-23% were only found either in LoFi or HiFi (Figure 2a). The mean number of MOTUs per sample was of 60 ± 13 in LoFi and 47 ± 11 in HiFi for *P. pinaster*, and of 55 ± 14 in LoFi and 44 ± 8 MOTUs in HiFi for *P. halepensis* (Table S3).

The 89.4 % of MOTUs were assigned to the phylum Basidiomycetes, the 10.2 % to Ascomycetes and the 0.4 % to Zygomycetes (Figure 2b; Table S4). The taxonomic classification of MOTUs allowed identifying 12 fungal orders, 30 families and 47 genera (Table S4). The majority of the top-20 most abundant fungi (Table 1) and of the indicator species (Table S5) found in root tips of *P. pinaster* belonged to *Tomentellaceae*, *Inocybaceae*, *Russulaceae*, and *Rhizopogonaceae* and, in the case of *P. halepensis*, to Pezizales, *Thelephoraceae* and *Sebacinaceae*. The indicator species analysis revealed 54 fungal MOTUs preferentially associated with *P. pinaster* and 37 with *P. halepensis* (Table S5). On the other hand, 31 distinct fungal MOTUs were LoFi indicators for both pine species, and 11 for *P. pinaster* and 6 for *P. halepensis* were high fire recurrence indicators (Table S5).

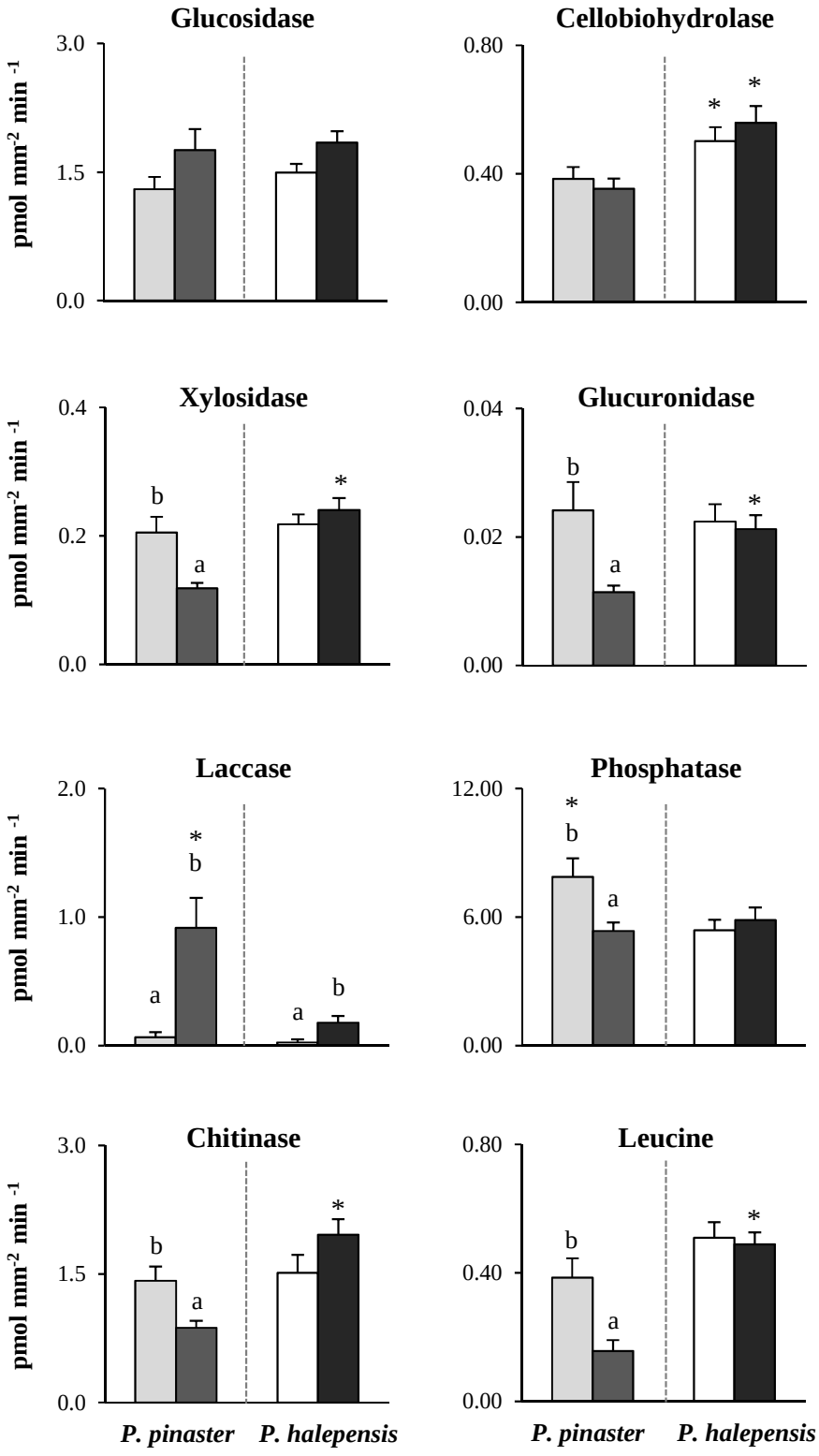

Figure 1 | Enzymatic activity of ectomycorrhizal root tips (means ± SE) of *Pinus pinaster* (Ppi) and *Pinus halepensis* (Pha) in response to different fire regimes: low (LoFi, light bars) and high (HiFi, dark bars) fire recurrence (serotinous populations), analysed by Generalized Linear Models ($p<0.05$). For each pine species, different letters denote significant differences between fire recurrence levels, while for each fire treatment, asterisks denote significant differences between pine species.

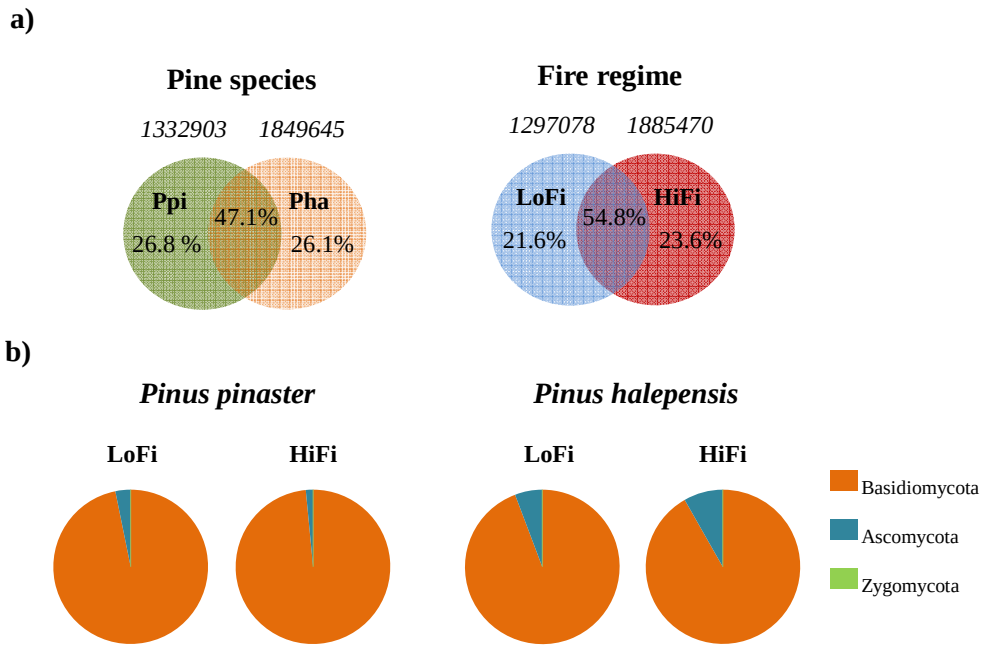

Figure 2 | (a) Number of sequences (*cursive*) and percentage of ectomycorrhizal fungal MOTUs by pine species (Ppi = *Pinus pinaster* and Pha = *Pinus halepensis*) and fire regime (LoFi = low, and HiFi = high fire recurrence). (b) Percentages of fungal phyla for each pine species and fire regime treatment.

Fungal community structure

Total fungal α-diversity did not vary between pine species, although it did by fungal phyla, with significantly higher number of Ascomycetes and lower of Basidiomycetes observed for *P. halepensis* compared with *P. pinaster* (Figure 3; Table S6). A negative effect of the high fire recurrence on ECM fungal α-diversity was evidenced for both pine species (Figure 3a; Table S6), and this effect was maintained when analysed by fungal phyla (Figure 3b-c; Table S6). In parallel, the pine species clearly determined the α-diversity of representative ECM fungal families (Table 2; Table S7). Compared with *P. halepensis*, *P. pinaster* root-tips were enriched of *Amanitaceae*, *Atheliaceae*, *Cantharellaceae*, *Clavariaceae*, *Cortinariaceae*, *Gloniaceae*, *Rhizopogonaceae* and *Russulaceae*, while for *P. halepensis* the families *Pezizaceae*, *Pyronemataceae* and *Sebacinaceae* were locally higher diverse (Table 2). The high fire recurrence decreased the α-diversity of many ECM fungal families (Table 2; Table S7), and this effect was maintained when analysed separately by pine species (Table 2). However, some fungal families were clearly favoured by fire, e.g. *Amanitaceae*, *Cantharellaceae* and *Rhizopogonaceae* for *P. pinaster*, and *Pezizaceae* in the case of *P. halepensis* (Table 2).

Table 1 | The 20-most abundant fungal MOTUs found in *Pinus pinaster* (Ppi) and *Pinus halepensis* (Pha) root tips. **¥ =** number of reads by pine species and fire regime (LoFi= low fire recurrence and HiFi= high fire recurrence).

BLAST identification	NCBI / UNITE	% id	E-value	Pine Species¥		Fire Regime	
				Ppi	Pha	LoFi	HiFi
P. pinaster							
Suillus bellinii	HM347655	100	1E-144	89643	15198	34171	70670
Craterellus lutescens	UDB011212	98	5E-128	87541	10093	16	97618
Tylospora sp.	KF007260	100	1E-119	80789	83	69703	11169
Rhizopogon verii	AM085531	99.2	0E-00	71158	11	12	71157
Amphinema sp.	HM146796	99	1E-136	48695	125	27732	21088
Inocybe mixtilis	JF908121	99	4E-155	35708	234	31893	4049
Russulaceae sp.	DQ061892	99	1E-168	35346	6180	20199	21327
Tomentella sp.	UDB018564	99	4E-156	33639	76899	61120	49418
Tomentella sp.	FJ210766	97.2	1E-130	31942	140486	45848	126580
Russula torulosa	UDB011110	100	1E-140	30952	58	25898	5112
Amphinema byssoides	JN943914	100	1E-119	27962	79119	20092	86989
Amphinema sp.	AB669503	99	1E-142	27809	181050	81935	126924
Rhizopogon graveolens f. pomaceus	AJ810037	99.3	1E-152	27400	9	3991	23418
Inocybe sp.	JQ975964	99.7	1E-167	26687	6	3705	22988
Inocybe mixtilis	JF908121	99	6E-147	26090	64	19976	6178
Tomentella coerulea	UDB003329	98.9	1E-140	24097	46119	28366	41850
Tomentella terrestris	UDB016369	99.6	1E-152	23053	649	12900	10802
Amphinema sp.	HM146796	97	8E-139	21642	465	5364	16743
Lactarius deliciosus	UDB002381	99.6	1E-156	21271	47	14334	6984
Russula sanguinea	UDB000899	99.6	1E-143	20794	2068	9936	12926
P. halepensis							
Amphinema sp.	HM146796	99	7E-146	27809	181050	81935	126924
Sebacina cystidiata	KF000452	98.2	1E-112	41	171441	41039	130443
Tomentella sp.	FJ210766	97.2	1E-130	31942	140486	45848	126580
Sebacina sp.	UDB009836	99.6	1E-129	21	82564	54754	27831
Amphinema byssoides	JN943914	100	1E-119	27962	79119	20092	86989
Tomentella sp.	UDB018564	99	4E-156	33639	76899	61120	49418
Tomentella sp.	EF507257	95	3E-138	10604	54800	48466	16938
Tomentella coerulea	UDB003329	98.9	1E-140	24097	46119	28366	41850
Tomentella sp.	HQ204742	98	1E-198	6	41684	41274	416
Tomentella sp.	KJ769318	97	8E-150	7	41062	17066	24003
Tricholoma batschii	UDB011579	99.3	1E-162	15468	40673	1619	54522
Sebacina sp.	UDB005787	97.9	1E-119	615	34632	9645	25602
Sebacina sp.	GU817065	100	1E-120	283	33761	20183	13861
Sebacina incrustans	EF644113	100	1E-123	3097	31355	31239	3213
Pseudotomentella sp.	UDB008306	97.9	1E-147	9	28708	447	28270
Sebacinaceae sp.	KF000650	97	6E-134	4	28200	6119	22085
Tomentella sp.	HE687163	99.6	1E-145	3327	26839	7	30159
Suillus collinitus	AY935517	100	5E-161	11	23715	376	23350
Tomentella sp.	AB211278	97	1E-131	3	23366	23338	31
Sebacina sp.	EF372401	99	1E-142	152	22590	895	21847

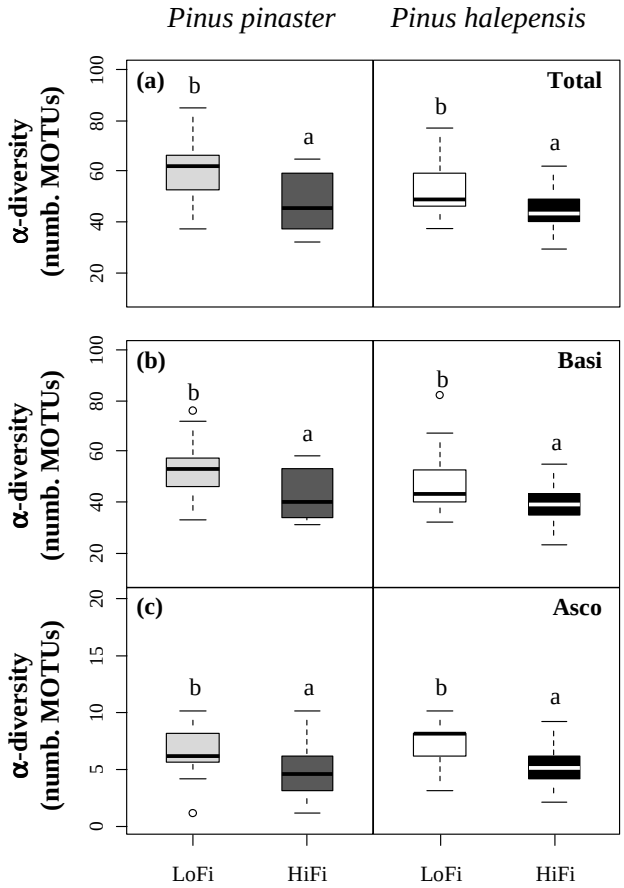

Figure 3 | Alpha-diversity of (a) total, (b) Basidiomycetes (Basi) and (c) Ascomycetes (Asco) fungal communities associated with root-tips of *Pinus pinaster* and *Pinus halepensis* in areas of low (LoFi, light bars) and high (HiFi, dark bars) fire recurrence, analysed by Generalized Linear Models ($p < 0.05$). Boxes represent the interquartile range (IQR) between first and third quartiles and the horizontal line inside is the median. Whiskers denote the lowest and highest values within 1.5 x IQR from the first and third quartiles, respectively. Within each graph, different letters denote significant differences among fire regimes.

Table 2 | Alpha-diversity of representative ECM fungal families analysed by General Linear Models ($p<0.05$). Main effects of the factors pine species (Ppi =*Pinus pinaster* and Pha =*Pinus halepensis*) and fire regime (LoFi = low recurrence and HiFi = high recurrence (serotinous populations)) (left), and separate analysis of fire regime effect by pine species (right). The site (i.e., pine population) was nested within the factor fire regime. Data = means +/- SE. Within each factor, arrows indicate significantly higher α-diversity. In the separate analyses, for each pine species, different letters denote significant differences between fire regime treatments ($p<0.05$) (in bold). A = Ascomycetes; B = Basidiomycetes (see Supplementary Table S7 for details).

	Pine species		**Fire regime**		***P. pinaster***		***P. halepensis***	
	Ppi	***Pha***	**LoFi**	**HiFi**	**LoFi**	**HiFi**	**LoFi**	**HiFi**
Amanitaceae^{B}	↑			↑	**0.1 ± 0.1 a**	**0.9 ± 0.2 b**	0.1 ± 0.1	0.2 ± 0.1
Atheliaceae^{B}	↑		↑		**9.7 ± 0.6 b**	**6.7 ± 0.6 a**	6.3 ± 0.5	5.9 ± 0.4
Bankeraceae^{B}	=	=	↑		1.8 ± 0.4	1.3 ± 0.3	**1.7 ± 0.5 b**	**0.9 ± 0.2 a**
Cantharellaceae^{B}	↑			↑	**1.0 ± 0.2 a**	**2.8 ± 0.4 b**	0.7 ± 0.2	0.5 ± 0.2
Clavariaceae^{B}	↑		=	=	0.8 ± 0.2	1.2 ± 0.4	**0.3 ± 0.2 b**	**0.1 ± 0.1 a**
Clavulinaceae^{B}	=	=	↑		**2.9 ± 0.3 b**	**1.7 ± 0.3 a**	**2.7 ± 0.5 b**	**1.3 ± 0.3 a**
Cortinariaceae^{B}	↑		↑		**2.2 ± 0.7 b**	**0.4 ± 0.2 a**	**0.6 ± 0.4 b**	**0.2 ± 0.1 a**
Gloniaceae^{A}	↑		↑		**2.5 ± 0.3 b**	**1.2 ± 0.2 a**	**1.7 ± 0.4 b**	**0.9 ± 0.3 a**
Hydnaceae^{B}	=	=	↑		0.3 ± 0.1	0.2 ± 0.1	**0.6 ± 0.2 b**	**0.2 ± 0.1 a**
Inocybeaceae^{B}	=	=	↑		**5.5 ± 0.8 b**	**3.0 ± 0.5 a**	3.1 ± 0.3	2.8 ± 0.3
Pezizaceae^{A}		↑		↑	0.2 ± 0.1	0.2 ± 0.1	**0.5 ± 0.2 a**	**1.1 ± 0.2 b**
Pyronemataceae^{A}		↑	↑		0.6 ± 0.2	0.9 ± 0.2	**2.9 ± 0.3 b**	**1.6 ± 0.3 a**
Rhizopogonaceae^{B}	↑		=	=	**0.9 ± 0.2 a**	**1.8 ± 0.2 b**	**0.8 ± 0.2 b**	**0.4 ± 0.1 a**
Russulaceae^{B}	↑		=	=	**7.3 ± 0.6 b**	**5.5 ± 0.7 a**	4.3 ± 0.8	4.3 ± 0.5
Sebacinaceae^{B}		↑	=	=	5.7 ± 1.1	4.5 ± 0.4	8.7 ± 0.8	9.0 ± 0.8
Suillaceae^{B}	=	=	↑		1.6 ± 0.3	1.2 ± 0.2	**1.6 ± 0.2 b**	**1.2 ± 0.2 a**
Telephoraceae^{B}	=	=	↑		11.9 ± 1.0	11.0 ± 1.0	**14.1 ± 1.8 b**	**10.4 ± 0.8 a**
Tuberaceae^{A}	=	=	=	=	1.4 ± 0.3	1.6 ± 0.3	1.6 ± 0.3	1.4 ± 0.2

At a regional scale, total ECM fungal β-diversity was not affected by the pine species (Table S6; Figure 4a). However, when analysed by phyla, Basidiomycetes were significantly more dissimilar in *P. pinaster* than in *P. halepensis* populations, while for Ascomycetes the opposite pattern was observed (Figure 4b-c). In any case, for both pine species the high fire recurrence caused a reduction of the ECM fungal β-diversity of root tips (i.e., more homogeneous communities) (Figure 4a; Table S6). Likewise, Basidiomycetes were less β-diverse in HiFi populations, while no difference was observed for Ascomycetes (Figure 4b-c; Table S6). The fire regime significantly affected the assemblage of the ECM fungal communities on root-tips of both pine species (Figure 5; Table S6). In both cases, the fungal species assemblage significantly correlated with soil pH and, in the case of *P. halepensis* also with P (Figure 5). The enzymatic activities significantly correlated with root-tip ECM fungal assemblages were related with the C cycle, i.e., glucosidase (for *P. pinaster*; Figure 5a) and xylosidase (for *P. halepensis*; Figure 5b),

and with N mobilization, i.e., chitinase and leucine for *P. pinaster* (Figure 5a) and leucine for *P. halepensis* (Figure 5b). Among pine species variables, root-tip fungal assemblages were separated by serotinous and not serotinous pine populations (i.e., closed cones) (Figure 5) and, for *P. pinaster*, also correlated with the productivity of the trees (diameter at breast height, DBH).

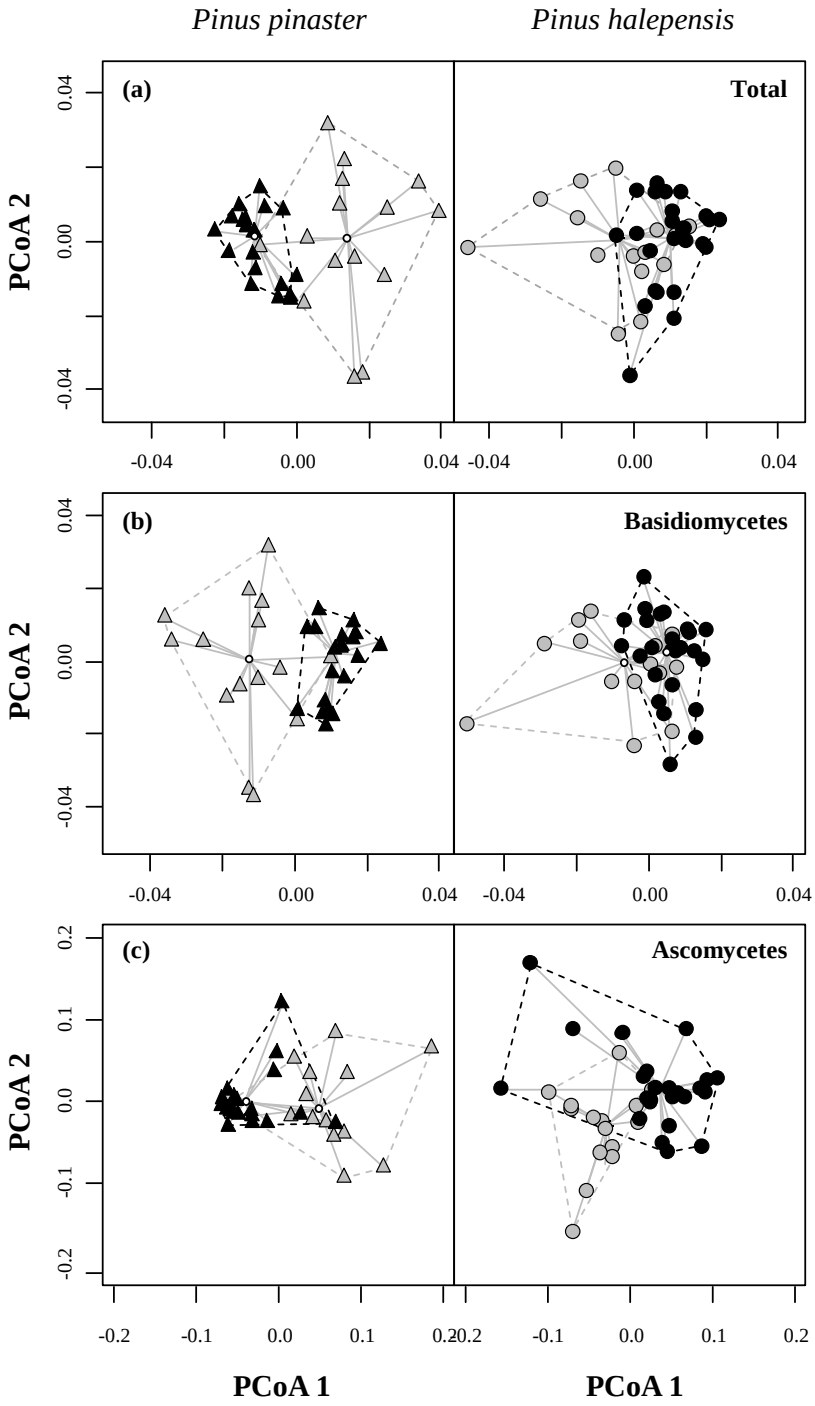

Figure 4 | Beta-diversity of (a) total, (b) Basidiomycetes (Basi) and (c) Ascomycetes (Asco) fungal communities associated with root-tips of *Pinus pinaster* (triangles) and *Pinus halepensis* (circles) in areas of low (LoFi, grey) and high (HiFi, black) fire recurrence The centroids within each group are represented by small white dots.

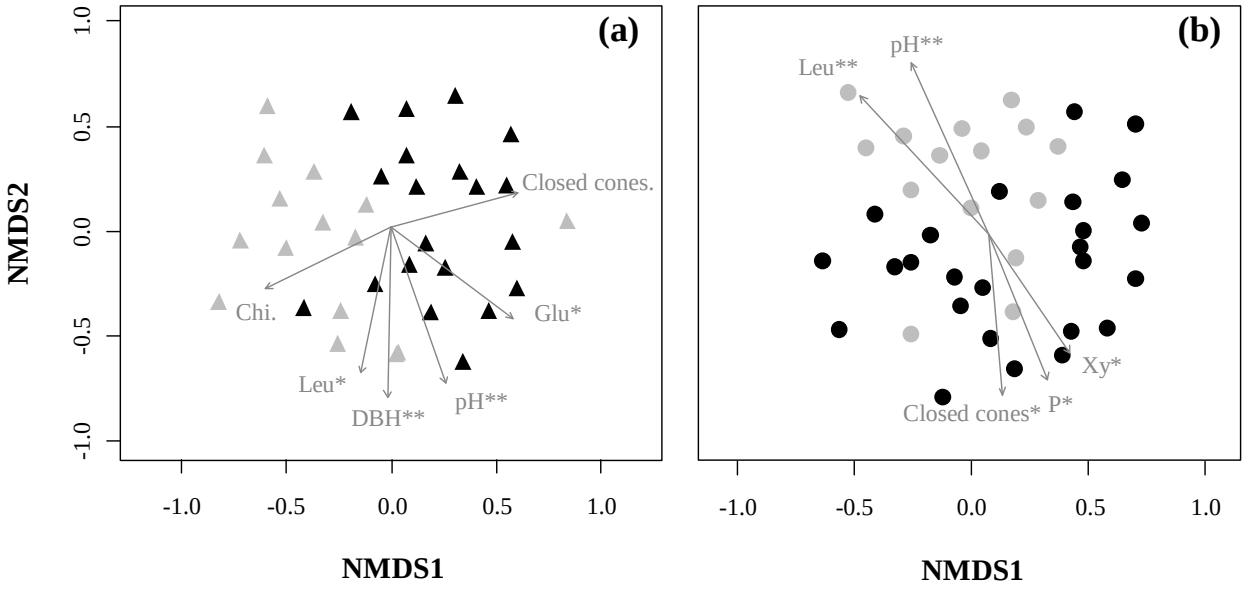

Figure 5 | Assemblage of ectomycorrhizal fungal MOTUs on root-tips of (a) *Pinus pinaster* and (b) *Pinus halepensis* by fire regime (grey = low fire recurrence; black = high fire recurrence (serotinous populations)). Vectors: strength and direction of tree variables weight (No. Closed cones = serotiny; DBH = diameter at breast height), soil variables (EC = electric conductivity; K = potassium; P = phosphate; OM = organic matter; N = nitrogen; C/N = carbon/nitrogen ratio) and enzymes (Glu = glucosidase; Xy = xylosidase; Chi = Chitinase; Leu = leucine) on the distribution of fungal MOTUs; *p<0.075; *p<0.05; **p <0.01; ***p<0.001. NMDS: k =2, stress=0.25, R^2=0.94 for (a) and (b) models.

DISCUSSION

Overall our results reveal that the fire regime is a key agent shaping the ECM fungal communities associated with root tips of two representative Mediterranean pines, *P. pinaster* and *P. halepensis*, at local and regional scales. Serotinous populations of both pines shelter less ECM fungal diversity than pine populations growing in areas of low fire recurrence, where the enzymatic activity is also greater, particularly in the case of *P. pinaster*. Both pine species harbor similarly enriched ECM fungal communities on root-tips, but different species assemblages, which also diverge in their functional response to the fire regime. Main functional adjustments on root-tip ECM fungal communities, related with structural shifts mediated by high fire recurrence and/or serotiny, are linked with increased carbon turnover and reduced mobilization of nitrogen.

ECM communities associated with root tips of *P. pinaster* and *P. halepensis*

Because of the known-role of tree species structuring fungal communities (Aponte *et al.*, 2010; Barbi *et al.*, 2016; Garcia *et al.*, 2016), and probably due to the contrasted local environment (see Chapter 5), different ECM fungal assemblages were associated with root-tips of *P. pinaster* and *P. halepensis*. Opposite to other mutualistic relationships, the ectomycorrhizal symbiosis is low specific, e.g., for *Pinus* spp. it can reach the genus level (Smith and Read, 2008). In fact, half of fungi were shared by both pine species, and fungal diversity did not vary between them, either at local or regional scales. However, the diversity of large fungal phyla did vary, with greater number and more heterogeneous Ascomycetes species together with less and more homogeneous Basidiomycetes in the case of *P. halepensis*, while the opposite happened for *P. pinaster*. These divergences could be mediated by the tree host species identity and/or by fungal fitness requirements for adaptation to the environment. In fact, the pH was pivotal shaping root-tip ECM fungal assemblages of both pine species, as often described (Rousk *et al.*, 2010; Coince *et al.*, 2014; Tedersoo *et al.*, 2014; Rincón *et al.*, 2015). It is possible that together with climate limitations, the bedrock influence, mainly siliceous for *P. pinaster*, and calcareous for *P. halepensis*, were among the strongest environmental filters for their associated ECM fungal communities affecting their fitness. In this sense, it is highlighting the observed preferential association of *P. halepensis* with fungal species of the genus *Sebacina* and the order Pezizales. Indeed, any tree host specificity has been observed among the *Sebacina* species (Selosse *et al.*, 2002; Ray and Craven, 2016), but given the reported positive effects of Sebacinales fungi against stresses such as herbivore, salinity or drought (Barazani and Baldwin, 2013; Zarea *et al.*, 2014; Ray and Craven, 2016), this genus could play a key role in the resistance of *P. halepensis* to limited nutrient availability (i.e., related to high soil pH) and the restrictive conditions imposed by the Mediterranean climate. Similarly, ectomycorrhizal Pezizales, which tend to be favoured in

basic soils, present traits that make them perfect symbionts under stressful conditions (Tedersoo *et al.*, 2006; Lamit *et al.*, 2016). In this sense, many ECM Ascomycetes produce cell wall melanin, which is a trait directly related to environmental stresses (Koide *et al.*, 2014; Treseder and Lennon, 2015). On the other hand, species from the genus *Rhizopogon*, *Craterellus*, *Cenoccocum*, *Russula* and *Lactarius* were enriched under *P. pinaster*. Although ECM fungi tolerate a wide range of pH (Rousk *et al.*, 2010), it has been shown that most of these fungi growth better under acidophilic conditions (Yamanaka, 2003). Accordingly, van der Heijden and Vosatka (1999) showed that species of *Lactarius*, *Russula* or *Cenoccocum* were more abundant in acidic sites, as those with siliceous soils in our study.

The fire regime filters the diversity and functioning of root-tip ECM fungal communities

In all cases, the local and regional diversity of root-tip ECM fungi significantly declined with the increased fire recurrence. Many fire studies have ascribed these patterns to the reduction of heat resistant propagules, the scarcity of suitable hosts, the direct burning of mycelium and roots, and/or the disturbed soil properties (Rincón and Pueyo, 2010; Holden *et al.*, 2013). Likewise, Glassman et al. (2016) showed that after severe fires, the fire-mediated decrease of ECM fungal diversity in the soil spores bank occurred through the elimination of rare species. In our study, the high fire recurrence caused a change in the species composition of the root-tip ECM fungal community, with more diverse and greater numbers of LoFi than HiFi indicator fungi. With all these premises, our results may indicate a simplification of the ECM fungal community within root systems due to the high selective pressure caused by recurrent fire. Different authors have proposed that in ecosystems subjected to frequent fires, the positive response of microorganisms would indicate a selection of the fire-tolerant ones over time (Dooley and Treseder, 2011; Rincón *et al.*, 2014; Buscardo *et al.*, 2015). Many studies in fire-prone forests based on chronosequences showed that ECM richness increase as time passes since the last fire (Dooley and Treseder 2011; Kipfer et al. 2011; Rincón et al. 2014; Sun et al. 2015), similar to that reported in studies of fugal species succession where it increases with the forest age (Twieg *et al.*, 2007). In same way, these studies highlight the importance of dispersion processes since the richness increase occurs by the integration of new fungal species. Similarly, in our study it is possible that the high fire recurrence has altered the equilibrium of root-tip ECM fungal diversity not being yet restored, and that fire-prone fungi as well as other pioneer species have benefited from the reduced competition induced by fire (Baar *et al.*, 1999; Smith *et al.*, 2004; Martín-Pinto *et al.*, 2006a; Buscardo *et al.*, 2010; Rincón *et al.*, 2014). However, typical late-stage species such as *Amanita* or *Lactarius* (Taylor and Bruns, 1999; Cairney and Chambers, 2013) also appeared as indicators of high fire recurrence. We suggest that the ecosystem equilibrium changes for better

adapting to the adverse conditions imposed by recurrent fire, and that the pre-fire conditions barely will be the same. Moreover, as for serotiny, it could be possible that the fire recurrence affects other tree traits, inducing for example a poorer root development. This could consecutively affect its associated ECM fungi by the reduction of the space for fungal colonization, and mechanisms such as priority effects could gain importance (Kennedy *et al.*, 2009; Peay *et al.*, 2012).

Similar to that described for plant communities (Verdú and Pausas, 2007; Pausas and Verdú, 2008), and given the decline in ECM fungal β-diversity, the habitat filtering was likely the dominant ecological process assembling root-tip ECM fungal communities under high fire recurrence, while without the fire pressure (i.e., low fire recurrence) competition among fungi could prevail. Given the importance of the tree host influence (Chapters 2-4), it is also highly plausible that indirect plant-mediated effects of high fire recurrence happened. In fact, the serotinous character of trees (higher under HiFi populations) shaped the ECM fungal communities for both pine species. It may be that the energy investment by trees to maintain serotinous cones (Groom and Lamont, 1997) would produce a redistribution of the carbon and consecutively select certain ECM fungi in their roots through a dissimilar organic input delivery (quantity and quality). In this sense, it would be reasonable to think that the mechanisms by which fire recurrence affects the ECM communities will depend on the edaphic compartment where these fungi settle, as it has been previously suggested (Rincón *et al.*, 2015; Goldmann *et al.*, 2016; Moeller and Peay, 2016). So that the root-tip ECM community, tightly linked the host, could be less "environment-dependent" than the one spreading as extramatrical mycelium in the soil, where other ecological fungal guilds strongly compete (i.e., saprotrophs). Additionally, it cannot be ruled out that the plant community composition of these forest (i.e., ericoid shrubs) could have play an important role in the restoration and composition of the global ECM fungal community (Torres and Honrubia, 1997; Martín-Pinto *et al.*, 2006b; Dean *et al.*, 2014; Buscardo *et al.*, 2015).

The increased fire recurrence affected the ecosystem functions by reducing most enzymatic activities in the case of *P. pinaster*, but not for *P. halepensis*. In fact, most of these activities were similar in LoFi populations of *P. pinaster* and *P. halepensis*. Given that as exposed above, both pine species shelter divergent ECM fungal communities, our results may indicate a functional complementarity of the communities not subjected to environmental stress (Jones *et al.*, 2010). At the same time, structural shifts mediated by high fire recurrence and/or serotiny on root-tip ECM fungal communities were related to increased carbon turnover and reduced nitrogen mobilization. Some studies based on fire chronosequences have pointed out the importance of the elapsed time for the recovery of soil enzymatic activities, which it is probably related to the restoration of the plant community (Holden *et al.*, 2013; Köster *et al.*, 2016). We observed that the

laccase activity secreted by ECM fungi, that controls the degradation of more recalcitrant C compounds (i.e., lignin), increased in HiFi populations of both forests, giving evidences of possible fire-related effects on litter chemistry over time. Moreover, in previous Chapter 5, we observed that the organic matter quality changed in HiFi soils from forests of both pine species. The litter stoichiometry, the availability of resources in the surrounding soil, and the tree host genetics are key factors affecting the enzymatic activity of ECM fungal communities (Courty *et al.*, 2011, 2016; Schneider *et al.*, 2012). Thus, we argue that as recurrent fires shape the plant community (serotiny), it may change the quality and quantity of the organic inputs delivered belowground. That could imply ECM fungal shifts, as well as more recalcitrant organic forms, which depending on the local conditions would affect the functional traits of ECM fungal communities. In a 2-million-year chronosequence, Albornoz et al. (2016) have recently shown strong variations of ECM fungal communities even within the same hosts, attributable not only to short-term fungal edaphic specialization or different inoculum density and composition, but also likely to longer-term ecosystem-level feedbacks among soil, plants and ECM fungi during pedogenesis.

Since fire is a main ecological factor in the Mediterranean area and given the importance of different ECM fungal assemblages on post-fire forest recovery (Pena and Polle, 2014), the observed fire regime-related structural and functional shifts in these communities could have essential implications for the resilience of Mediterranean forest ecosystems. Better understanding of fungal-biotic-abiotic interactions under environmental stresses e.g., increased fire recurrence, would allow us to make predictions to face the new climate scenarios and to promote a sustainable management of Mediterranean forest ecosystems.

SUPPORTING INFORMATION

Table S1 | Characteristics of the pine populations (*Pinus pinaster* and *Pinus halepensis*) and sites studied, including the fire regime (LoFi = low recurrence; not serotinous populations) and HiFi = high recurrence; serotinous populations), geographical coordinates (latitude and longitude, ^{o}), altitude (m), total annual precipitation (Precip, mm), mean annual temperature (T^{a}, ^{o} C), and means ± SD per populations of pH, diameter at breast height (DBH, cm), bark thickness (cm) and number of closed cones (conesC) (serotiny indicator); n = 5 trees per site. Geographical coordinates, altitude, precipitation and temperature data are extracted from Hernández-Serrano et al. (2013).

Fire regime	Population	Lat/Lon, ^{o}	Altitude	Precip.	T^{a}	pH	DBH	Bark	ConesC
P. pinaster									
LoFi	Olba	40.17, -0.62	986	591	12.7	5.5 ± 0.5	27.8 ± 2.6	2.3 ± 0.2	0 ± 0
LoFi	Penyagolosa	40.25, -0.35	1365	682	11.3	5.1 ± 0.5	30.5 ± 2.9	2.6 ± 0.2	0 ± 0
LoFi	Sinarcas	39.79, -1.20	890	468	13.1	7.0 ± 0.6	33.3 ± 4.3	3.2 ± 0.5	1 ± 2
HiFi	Serra Calderona	39.75, -0.50	810	582	13.4	5.9 ± 0.2	25.2 ± 3.7	3.6 ± 0.2	23 ± 11
HiFi	Eslida	39.88, -0.30	440	580	15.2	5.4 ± 0.3	29.3 ± 1.8	3.1 ± 0.5	15 ± 5
HiFi	Pobla Tornesa	40.08, 0.01	474	644	15.0	6.0 ± 0.8	20.8 ± 3.8	2.9 ± 1.1	22 ± 14
HiFi	Quatretonda	38.97, -0.36	463	547	15.1	6.2 ± 0.6	35.4 ± 5.3	3.8 ± 0.4	10 ± 3
P. halepensis									
LoFi	Montan	40.05, -0.59	900	584	13.0	8.0 ± 0.0	26.2 ± 3.2	2.5 ± 0.2	6 ± 1
LoFi	Sinarcas	39.80, -1.20	913	471	13.0	7.1 ± 0.4	28.2 ± 1.4	3.0 ± 0.4	3 ± 2
LoFi	Titaguas	39.89, -1.30	880	452	13.1	7.8 ± 0.1	25.1 ± 3.7	2.5 ± 0.4	5 ± 3
HiFi	Alzira	39.12, -0.39	147	511	16.9	6.9 ± 0.5	27.2 ± 4.0	2.7 ± 0.4	30 ± 9
HiFi	Cabanes	40.10, 0.04	445	647	15.2	6.9 ± 0.6	31.3 ± 6.2	2.8 ± 0.3	20 ± 7
HiFi	Serra Calderona	39.74, -0.48	706	574	13.9	7.6 ± 0.3	33.3 ± 3.7	3.1 ± 0.3	34 ± 5
HiFi	Eslida	39.87, -0.29	510	589	14.9	7.5 ± 0.5	32.0 ± 3.1	2.8 ± 0.4	19 ± 2
HiFi	Serra d'Irta	40.35, 0.32	347	692	15.7	6.9 ± 0.5	27.3 ± 4.1	2.7 ± 0.2	17 ± 7

Table S2 | Enzymatic activity of ectomycorrhizal root tips of *Pinus pinaster* and *Pinus halepensis*. Effects of (a) the fire regime (low and high fire recurrence, i.e., not serotinous and serotinous pine populations, respectively), the pine species, and their interaction, and (b) separate effect of the fire regime (LoFi and HiFi treatments) by pine species, analysed by General Linear Models. df = degrees of freedom; F-values, and asterisks denote *p*-value: ns = not significant; .p<0.08; *p<0.05; **p<0.01;***p<0.001.

		Carbon cycle					P cycle	N cycle	
	df	Glucosidase	Cellobiohydrolase	Xylosidase	Glucuronidase	Laccase	Phosphatase	Chitinase	Leucine
(a) Main test									
Fire	1	4.09*	0.07^{ns}	3.63.	8.28**	18.63***	3.05^{ns}	0.02^{ns}	8.20**
Pine species	1	1.63^{ns}	13.6***	23.09***	6.16*	15.79***	1.57^{ns}	17.72***	43.60***
Fire x Species	1	0.23^{ns}	0.26^{ns}	1.03^{ns}	0.17^{ns}	1.03^{ns}	1.37^{ns}	1.63^{ns}	0.82^{ns}
(b) Fire effect by pine species									
P. pinaster	1	1.64^{ns}	0.45^{ns}	13.76***	12.66**	15.77***	8.89**	8.39**	17.00***
P. halepensis	1	3.32^{ns}	0.32^{ns}	0.27^{ns}	0.46^{ns}	4.70*	0.34^{ns}	2.75^{ns}	0.05^{ns}

Table S3 | Summary of sequencing yields for the ectomycorrhizal fungal community of root-tips: (a) total, (b) per pine species (Ppi = *Pinus pinaster* and Pha = *Pinus halepensis*), (c) per fire regime (LoFi = low fire recurrence, HiFi = high fire recurrence), and (d) per pine species and fire regime.

(a) Total

TOTAL	Sequences	MOTUs
Total (n = 75)	3182548	501
per sample (mean ± SD)	42434 ± 11366	50 ± 13

(b) per Pine species

	Nb. Reads	MOTUs
Ppi (n = 35)	1332903	370
Pha (n = 40)	1849645	367
Ppi (Mean ± SD**)**	38083 ± 11857	53 ± 14
Pha (Mean ± SD**)**	46241 ± 9523	48 ± 12

(c) per Fire recurrence

	Nb. Reads	MOTUs
LoFi (n = 30)	1297078	383
HiFi (n = 45)	1885470	393
LoFi (Mean ± SD**)**	43236 ± 11816	57 ± 14
HiFi (Mean ± SD**)**	41899 ± 11158	45 ± 10

(d) per Pine species and Fire recurrence

	Pinus pinaster		***Pinus halepensis***	
Fire recurrence	**Nb. Reads**	**MOTUs**	**Nb. Reads**	**MOTUs**
LoFi (n = 15)	596207	281	700871	253
HiFi (n = 20/25)	736696	262	1148774	290
LoFi (Mean ± SD**)**	39747 ± 14082	60 ± 13	46725 ± 8052	55 ± 14
HiFi (Mean ± SD**)**	36835 ± 10081	47 ± 11	45951 ± 10455	44 ± 8

Table S4 | Ectomycorrhizal fungal MOTUs assigned to phylum, order, family and genus per pine species and fire regime (LoFi = low fire recurrence, and HiFi = high fire recurrence (serotinous pine populations).

PHYLUM (3)		*Pinus pinaster*		*Pinus halepensis*	
	Total	**LoFi**	**HiFi**	**LoFi**	**HiFi**
Basidiomycota	448	332	230	224	251
Ascomycota	51	36	31	29	39
Zygomycota	2	2	1	0	0
Total MOTUs	501	370	262	253	290

ORDER (12)		*Pinus pinaster*		*Pinus halepensis*	
	Total	**LoFi**	**HiFi**	**LoFi**	**HiFi**
Agaricales	103	77	41	41	43
Atheliales	37	31	21	16	17
Boletales	19	14	11	10	11
Cantharellales	35	31	24	17	19
Endogonales	2	2	1	0	0
Gomphales	2	1	1	2	1
Hysterangiales	3	2	0	0	1
Hysteriales	6	5	4	5	6
Incertae sedis	3	3	2	1	1
Pezizales	42	28	25	23	32
Russulales	61	41	31	25	38
Sebacinales	44	35	26	29	34
Thelephorales	144	100	75	84	87

FAMILY (30)		*Pinus pinaster*		*Pinus halepensis*	
	Total	**LoFi**	**HiFi**	**LoFi**	**HiFi**
Albatrellaceae	2	2	1	0	0
Amanitaceae	5	4	3	1	3
Atheliaceae	37	31	21	16	17
Bankeraceae	15	10	6	11	8
Boletaceae	1	1	1	1	0
Cantharellaceae	10	10	8	4	4
Clavulinaceae	18	16	13	10	12
Cortinariaceae	23	21	7	7	3
Discinaceae	1	1	0	0	0
Endogonaceae	2	2	1	0	0
Gloniaceae	6	5	4	5	6
Gomphaceae	2	1	1	2	1
Gomphidiaceae	2	1	1	1	0
Hydnaceae	7	5	3	3	3
Hydnangiaceae	1	1	1	0	0
Hygrophoraceae	5	5	2	3	2
Hysterangiaceae	3	2	0	0	1
Incertae sedis	3	3	2	1	1
Inocybaceae	53	35	21	23	27
Paxillaceae	1	0	0	0	1
Pezizaceae	8	4	3	3	5
Pyronemataceae	20	11	10	13	16
Rhizopogonaceae	6	5	5	3	4
Russulaceae	59	39	30	25	38
Sclerodermataceae	3	1	1	1	1
Sebacinaceae	44	35	26	29	34
Strophariaceae	4	3	2	2	0
Suillaceae	6	6	3	4	5
Thelephoraceae	129	90	69	73	79
Tricholomataceae	12	8	5	5	8
Tuberaceae	13	12	12	7	11

GENUS (47)		*Pinus pinaster*		*Pinus halepensis*	
	Total	**LoFi**	**HiFi**	**LoFi**	**HiFi**
Amanita	5	4	3	1	3
Amphinema	22	16	13	13	14
Boletopsis	1	0	0	1	1
Boletus	1	1	1	1	0
Brauniellula	1	0	0	1	0
Cenococcum	6	5	4	5	6
Chroogomphus	1	1	1	0	0
Clavulina	14	13	12	9	9
Cortinarius	22	20	7	7	3
Craterellus	5	5	5	3	4
Endogone	2	2	1	0	0
Genabea	2	0	0	2	1
Genea	1	0	0	0	1
Geopora	12	7	6	8	11
Hebeloma	4	3	2	2	0
Hydnellum	10	7	6	8	6
Hydnotrya	1	1	0	0	0
Hydnum	7	5	3	3	3
Hygrophorus	5	5	2	3	2
Hysterangium	3	2	0	0	1
Inocybe	53	35	21	23	27
Laccaria	1	1	1	0	0
Lactarius	9	8	7	4	5
Macowanites	2	1	1	1	2
Melanogaster	1	0	0	0	1
Meliniomyces	3	3	2	1	1
Phaeoclavulina	1	0	0	1	0
Phellodon	3	2	0	2	1
Piloderma	9	9	3	2	2
Pseudotomentella	8	7	6	7	4
Ramaria	1	1	1	1	1
Rhizopogon	6	5	5	3	4
Russula	42	26	20	17	26
Sarcodon	1	1	0	0	0
Sarcosphaera	3	2	1	2	2
Scleroderma	3	1	1	1	1
Sebacina	34	27	21	21	25
Suillus	6	6	3	4	5
Terfezia	4	2	2	0	3
Thelephora	4	1	1	4	1
Tomentella	109	75	55	59	72
Tomentellopsis	8	7	7	3	2
Tricholoma	12	8	5	5	8
Trichophaea	2	1	1	0	1
Tuber	13	12	12	7	11
Tylospora	6	6	5	1	1
Wilcoxina	2	2	2	2	1

Table S5 | Indicator fungal species at a significant level of $p \leq 0.05$ and their abundances (number of reads) associated with (a) different pine species (Ppi = *Pinus pinaste*r and Pha = *Pinus halepensis*), and (b) different fire regime (LoFi = low fire recurrence, and HiFi = high fire recurrence (serotinous populations).

(a) By Pine species

Pinus pinaster	***p***	**Ppi**	**Pha**
Tylospora sp.	0.001	80789	83
Amphinema sp.	0.001	48695	125
Suillus bellinii sp.	0.001	89643	15198
Rhizopogon graveolens f. pomaceus	0.001	27400	9
Meliniomyces bicolor sp.	0.001	10205	28
Hydnellum auratile	0.001	16162	20
Craterellus lutescens	0.006	87541	10093
Russula torulosa	0.001	30952	58
Lactarius deliciosus	0.001	21271	47
Tylospora sp.	0.001	227	0
Rhizopogon sp.	0.001	71158	11
Inocybe mixtilis	0.01	35708	234
Inocybe mixtilis	0.001	26090	64
Tylospora sp.	0.001	339	0
Craterellus lutescens	0.001	15854	2056
Tylospora sp.	0.001	5358	0
Tomentella sp.	0.001	18450	3
Inocybe amethystina	0.02	17632	1461
Tylospora sp.	0.001	235	0
Cenococcum sp.	0.015	414	73
Russula sp.	0.001	12712	0
Lactarius torminosus	0.001	17875	2
Tomentella sp.	0.01	2572	15
Inocybe sp.	0.006	4584	118
Tomentella sp.	0.019	17073	2302
Tomentella subclavigera	0.001	5561	0
Lactarius chrysorrheus	0.001	5092	0
Craterellus lutescens	0.001	143	0
Meliniomyces bicolor	0.002	104	0
Craterellus lutescens	0.002	1188	73
Clavulina sp.	0.002	3297	1
Russula laricina	0.003	14901	174
Russula sanguinea	0.04	20794	2068
Russula sp.	0.004	3624	0
Russula sp.	0.048	7387	7
Piloderma sp.	0.009	6071	0
Hydnellum auratile	0.005	200	0
Russula sp.	0.031	7117	2
Sebacina sp.	0.021	591	2
Inocybe lilacina	0.034	1411	48
Inocybe posterula	0.015	2513	0
Inocybe sororia	0.023	1731	0
Tomentella badia	0.021	1358	0
Albatrellaceae sp.	0.019	1332	0
Piloderma sp.	0.014	1254	0
Tricholoma albobrunneum	0.021	462	0
Tomentellopsis echinospora	0.029	276	0
Russula sp.	0.02	239	0
Russula laricina	0.049	137	2
Hebeloma cistophilum	0.044	1782	0
Tomentellopsis sp.	0.043	951	0
Inocybe leiocephala	0.031	904	0
Amanita sp.	0.038	318	0
Cantharellaceae sp.	0.044	139	0

Pinus halepensis	***p***	**Ppi**	**Pha**
Sebacina sp.	0.001	41	171441
Amphinema sp.	0.001	0	907
Sebacina sp.	0.006	21	82564
Sebacina sp.	0.001	615	34632
Lactarius sanguifluus	0.003	268	20393
Sebacinaceae sp.	0.001	4	28200
Suillus collinitus	0.001	11	23715
Pseudotomentella atrofusca	0.004	88	12728
Sebacina sp.	0.001	665	8936
Sebacina sp.	0.003	152	22590
Tomentella sp.	0.005	6	41684
Tuber maculatum	0.001	3	15875
Pseudotomentella sp.	0.004	9	28708
Sebacina sp.	0.001	0	1717
Tomentella sp.	0.013	7	41062
Sebacina sp.	0.026	283	33761
Terfezia sp.	0.002	2	16960
Sebacina sp.	0.026	502	13483
Sebacina sp.	0.001	0	398
Amphinema sp.	0.003	5	162
Geopora sp.	0.005	0	5939
Inocybe sp.	0.042	4	19263
Geopora sp.	0.01	4	9299
Sebacinaceae sp.	0.002	8	1506
Tricholoma terreum	0.014	252	11355
Geopora sp.	0.028	2	6548
Sebacina sp.	0.006	2	157
Genabea sphaerospora	0.009	0	9744
Sebacinaceae sp.	0.011	0	4033
Suillus mediterraneensis	0.032	71	7007
Inocybe griseotarda	0.019	1	14765
Sarcosphaera coronaria	0.008	1	1828
Hydnellum sp.	0.047	2	2661
Russulaceae sp.	0.05	16	1675
Tomentella sp.	0.015	0	10772
Geopora sp.	0.023	0	1819
Tomentella sp.	0.028	0	159

Continuation **Table S5**

(b) By Pine species and Fire Regime

(a) *Pinus pinaster*		*Reads*	
LoFi	***p***	**LoFi**	**HiFi**
Meliniomyces bicolor	0.001	10113	92
Cenococcum sp.	0.004	1948	25
Tylospora sp.	0.003	69632	11157
Tomentella sp.	0.004	31879	1760
Inocybe mixtilis	0.003	31665	4043
Tomentella sp.	0.002	18446	4
Clavulina sp.	0.001	338	2
Inocybe mixtilis	0.012	19915	6175
Tuber borchii	0.047	12220	3956
Russula sp.	0.002	12709	3
Clavulina sp.	0.002	1219	27
Tomentella sp.	0.009	16530	543
Cenococcum sp.	0.007	615	32
Meliniomyces bicolor	0.004	98	6
Russula laricina	0.002	14899	2
Tylospora sp.	0.039	282	57
Clavulina sp.	0.003	184	4
Tylospora sp.	0.027	163	72
Tomentella sp.	0.004	14457	1
Tomentella sp.	0.004	6109	2
Wilcoxina rehmii	0.015	2617	2
Cortinarius sp.	0.01	4010	0
Piloderma sp.	0.009	1254	0
Russula laricina	0.008	137	0
Sebacina sp.	0.028	859	2
Macowanites vinaceodorus	0.037	810	25
Inocybe leiocephala	0.027	904	0
Cortinarius sp.	0.026	509	0
Pseudotomentella atrofusca	0.033	237	0
Piloderma olivaceum	0.026	5805	1
Inocybe posterula	0.022	2512	1
HiFi	***p***	**LoFi**	**HiFi**
Craterellus lutescens	0.002	6	87535
Rhizopogon sp.	0.007	6	71152
Craterellus lutescens	0.006	3	15851
Tomentella sp.	0.01	5	3322
Tomentella sp.	0.003	107	11605
Craterellus lutescens	0.006	0	1188
Craterellus lutescens	0.006	0	143
Lactarius torminosus	0.017	26	17849
Tomentella sp.	0.012	42	2530
Lactarius chrysorrheus	0.015	2	5090
Amanita phalloides	0.023	0	5142

(b) *Pinus halepensis*		*Reads*	
LoFi	***p***	**LoFi**	**HiFi**
Clavulina sp.	0.001	482	124
Russulaceae sp.	0.014	5239	941
Genabea sphaerospora	0.001	9744	0
Tomentella sp.	0.009	41272	412
Suillus bellinii	0.011	12633	2565
Clavulina sp.	0.005	234	54
Clavulina sp.	0.001	75	9
Cenococcum sp.	0.004	4179	30
Geopora sp.	0.001	7564	1735
Sebacina sp.	0.016	9403	1266
Amphinema sp.	0.035	76	49
Sebacina incrustans	0.006	31236	119
Hydnum sp.	0.003	1277	0
Russula anthracina	0.003	7527	1
Tomentella cinerascens	0.002	8679	2
Inocybe sp.	0.032	19258	5
Suillus mediterraneensis	0.008	6992	15
Tomentella sp.	0.002	541	0
Inocybe griseotarda	0.041	14528	237
Tomentella sp.	0.002	154	5
Sarcosphaera coronaria	0.044	1456	372
Sebacina sp.	0.015	7290	0
Tomentella sp.	0.018	7	0
Tomentella cinerascens	0.014	474	0
Rhizopogon mohelnensis	0.031	6449	1
Tomentella sp.	0.027	453	4
Sebacinaceae sp.	0.036	857	10
Geopora sp.	0.048	1776	43
Tomentella sp.	0.047	1910	0
Tomentella sp.	0.046	843	0
Tuber melosporum	0.050	2563	1
HiFi	***p***	**LoFi**	**HiFi**
Suillus collinitus	0.013	372	23343
Lactarius sanguifluus	0.049	1269	19124
Sebacina sp.	0.013	853	21737
Terfezia sp.	0.008	0	16960
Amphinema sp.	0.021	5	157
Terfezia sp.	0.031	0	72

Table S6 | (a) Alpha-diversity, (b) Beta-diversity and (c) Assemblage of the total ectomycorrhizal fungal community and of that of representative fungal guilds, for the factors fire regime (LoFi = low fire recurrence and HiFi = high fire recurrence (serotinous populations)), pine species (Ppi = *Pinus pinaster* and Pha = *Pinus halepensis*) and their interaction, analysed by (a) General Linear Models (α -diversity), (b) Multivariate Homogeneity of Groups Dispersions (β-diversity), and (c) Permutation variance ADONIS analyses (assemblage).
In all cases, fire regime effect was also tested in separate analyses for each pine species. df = degrees of freedom. F and p-value : ns = not significant; *p<0.05; **p<0.01; ***p<0.001. BASI = Basidiomycetes; ASCO = Ascomycetes.

		TOTAL	BASI	ASCO
(a) α -diversity	df			
Fire	1	**25.37*** **	**21.44*** **	**12.38*** **
Pine species	1	2.66^{ns}	**4.96***	**4.72***
Fire x Sp	1	0.59^{ns}	0.33^{ns}	1.74^{ns}
Fire effect by pine species				
Ppi	1	**15.81*** **	**14.64*** **	**7.34***
Pha	1	**9.16****	**7.20***	**6.23***
(b) β-diversity				
Fire	1	**11.64****	**11.04****	1.47^{ns}
Pine species	1	1.34^{ns}	**4.44***	**11.62****
Fire x Sp	3	**3.56***	**4.51****	**4.91****
Fire effect by pine species				
Ppi	1	**6.34***	**5.95***	2.17^{ns}
Pha	1	**3.99***	**4.78***	0.01^{ns}
(c) Species Assemblage				
Fire	1	**5.69*** **	**5.27*** **	**5.27*** **
Pine species	1	**11.07*** **	**11.34*** **	**11.34*** **
Fire x Sp	1	**1.84***	**1.89****	**1.89***
Fire effect by pine species				
Ppi	1	**5.16*** **	**4.75*** **	**9.00*** **
Pha	1	**3.23*** **	**2.95*** **	**4.53*** **

Table S7 | Alpha-diversity of representative ectomycorrhizal fungal families analysed by General Linear Models ($p<0.05$). Main effects of the factors pine species (Ppi =*Pinus pinaster* and Pha =*Pinus halepensis*) and fire regime (LoFi = low fire recurrence, and HiFi = high fire recurrence (serotinous populations)) (left), and separate analysis of the fire regime effect by pine species (right). The site (i.e., pine population) was nested within the factor fire regime in models. F values; *$p<0.05$; **$p<0.01$; ***$p<0.001$. A = Ascomycetes; B = Basidiomycetes.

	Fire	***Species***	***Fire x Species***	Fire effect by pine species	
				P. pinaster	***P. halepensis***
Amanitaceae[B]	15.7***	9.2**	0.5	12.2***	3.3
Atheliaceae[B]	8.5**	6.8*	0.6	9.9**	0.8
Bankeraceae[B]	6.1*	2.0	1.2	1.6	4.1*
Cantharellaceae[B]	4.1*	41.7***	1.0	17.2***	1.0
Clavariaceae[B]	0.3	36.9***	0.1	0.7	6.8*
Clavulinaceae[B]	13.5***	2.4	0.0	7.0*	8.2**
Cortinariaceae[B]	23.2***	16.2***	12.4***	24.4***	5.8*
Gloniaceae[A]	18.1***	5.7*	0.2	14.4***	4.8*
Hydnaceae[B]	4.8*	0.8	0.9	1.1	4.3*
Inocybeaceae[B]	5.5*	1.4	5.4*	6.2*	0.4
Pezizaceae[A]	6.2*	22.8***	0.2	0.0	8.0**
Pyronemataceae[A]	3.9*	30.7***	0.2*	1.2	13.7***
Rhizopogonaceae[B]	0.0	29.1***	4.8*	8.7**	4.3*
Russulaceae[B]	2.9	14.0***	1.1	6.7*	0.0
Sebacinaceae[B]	0.2	33.6***	0.0	0.7	0.0
Suillaceae[B]	5.3*	0.0	0.1	1.2	4.6*
Telephoraceae[B]	4.2*	0.0	1.0	0.7	5.0*
Tuberaceae[A]	0.1	0.0	0.6	0.0	0.7

Chapter 7

Pine population genetics and fire regime shape the phylogenetic structure and functional traits of fungal communities in Mediterranean pine forests

INTRODUCTION

Fungal communities represent a significant fraction of forest ecosystems being directly involved in the biogeochemical cycling of nutrients and the productivity of trees (Smith and Read, 2008). Functional and phylogenetic distinct fungi differentially interact with plant species in forests, playing main roles in the dynamics of these ecosystems (Klironomos, 2003; Amend *et al.*, 2016). Fungi produce a wide set of extracellular enzymes able to decay biopolymers contained in the organic matter (Sinsabaugh, 2010; Baldrian, 2014) and, together with bacteria, they are main responsible for organic matter degradation (Cairney and Meharg, 2002; McGuire and Treseder, 2010). Soil enzymatic activity is a valuable indicator of the functional responses mediated by microbial and host nutrient requests (Olander and Vitousek, 2000; Allison and Vitousek, 2005). Soil fungal communities are of critical importance in maintaining the sustainability of ecosystems, and persistent disturbances, e.g., recurrent burning in Mediterranean forests, may shift their species composition causing substantial impacts on biogeochemical processes and ecosystem performance (Köster *et al.*, 2016; Shen *et al.*, 2016). Recurrent fires may affect the specific composition and functioning of fungal communities through the loss of vegetation and soil organic layer, and alterations of soil pH and fertility (Chen and Cairney, 2002; Hart *et al.*, 2005). Since belowground microorganisms are influenced by the plant community (Chapters 2, 3, 4, 6), besides direct effects of fire on soil and fungi, changes on trees are likely to strongly influence fungal communities during long-term post-fire secondary succession (Holden *et al.*, 2013; Sun *et al.*, 2015). Recent work has highlighted the important role of above-belowground interactions driving diversity patterns during ecosystem development (Fukami *et al.*, 2010; Martínez-García *et al.*, 2015), yet the structural and functional outcomes of plant–fungal feedbacks are less clear (Dickie *et al.*, 2013).

Mediterranean pines are clear examples of species shaped by fire (Pausas, 2015), e.g., *Pinus pinaster* Ait. and *Pinus halepen*sis Mill. These species show different ecological requirements (see Chapter 6), and are widely spread across the Mediterranean basin, usually with scattered distribution due to frequent ecological disturbances, i.e., fire (Gómez *et al.*, 2005). Because fire is an inherent ecological factor of the Mediterranean ecosystems, it is a main evolutionary force driving local adaptation and trait divergence (i.e., serotiny) in *P. pinaster* and *P. halepensis* forests (Hernández-Serrano *et al.*, 2013, 2014).

Phenotypic differences or similarities among species within a community are based in their evolutionary history, and it is commonly accepted that closely related species tend to be ecologically more similar than distantly related ones (Webb *et al.*, 2002). It has been demonstrated that recurrent burning induces the phylogenetic clustering of aboveground plant communities in Mediterranean ecosystems (Verdú and Pausas, 2007; Pausas and Verdú, 2008),

and similar effects could be expected on belowground fungal communities. In that case, fungal taxa with traits conferring the ability to survive fire and/or post-fire conditions would be environmentally filtered with potential functional consequences, as it has been suggested (Dooley and Treseder, 2011; Rincón *et al.*, 2014; Buscardo *et al.*, 2015). However, little is known as to how the assembly history (e.g., fire regime) and structural shifts of fungal communities may affect the overall ecosystem functioning (Fukami *et al.*, 2010). Additionally, beyond the tree species identity, even different tree genotypes and phenotype variants can directly influence their associated fungal communities (Gehring and Whitham 1991; van der Heijden et al. 2015; see Chapters 2-4), and the selection by fire of certain tree phenotypes can have a strong weight shaping the belowground fungal communities in the long-term (Hart *et al.*, 2005). The increasing availability of genomic resources in conifer species makes attracting approaches based on candidate gene sequencing (González-Martínez *et al.*, 2010), and provides an excellent opportunity to relate phylogenetic groups of fungi with their hosts.

In previous Chapter 6, we showed that root-tip ectomycorrhizal (ECM) fungal communities were shaped by the tree species identity and the fire regime, with functional variations and reduced fungal α-diversity under high fire recurrence. These effects could be attributed either to plant fire-adaptation (i.e., serotinous populations), local environmental conditions (e.g., soil properties), and/or to long-term fungal fire-adaptation. Trying to depict some of these possible mechanisms, in the current Chapter, we suggested that the pine population genetics would print a phylogenetic signal on their associated fungal communities, and that potential derived phylogenetic fungal shifts would entail functional responses under different fire regime. On the other hand, fire-induced effects on soil quality would affect the ecosystem functioning directly and through modulating the phylogenetic structure of fungal communities. Moreover, we comparatively studied root-tip and bulk soil fungal communities, to evaluate the strength of spatial distribution in the structural and functional responses of these communities.

In accordance with our previous results, we expected that i) the high fire recurrence would reduce the phylodiversity of root-tip and soil fungal communities (i.e., phylogenetic clustering), with functional consequences for carbon turnover and nutrient mobilization, and that ii) these structural and functional responses would diverge depending upon the tree species identity and genetics, the soil environment, and/or the edaphic compartment.

MATERIAL AND METHODS

Study area and sampling

The study area and the experimental design were the same than those described in previous Chapters 5 and 6. Natural populations of *P. pinaster* (Ppi) and *P. halepensis* (Pha) were selected in locations with different fire regimes in eastern Spain (Hernández-Serrano *et al.*, 2013). Nine populations were located in areas with historically frequent crown-fires (HiFi populations), while the other six populations were located in zones with low fire recurrence (LoFi populations) (Pausas *et al.*, 2004; Abdel Malak and Pausas, 2006; Verdú and Pausas, 2007; Pausas and Fernández-Muñoz, 2012) (see more details in Chapters 5 and 6).

The sampling was carried out in spring 2013, and five trees separated of more than 10 m were selected within each population (n = 75). Four samples located 1 m away of the trunk of each tree were collected in the four cardinal points, by digging 10 × 10 × 20 cm soil holes after removing the litter layer. The four sub-samples per tree were pooled into a single combined sample and kept at 4 ºC in plastic bags until processing. Once in the lab, roots were separated from soil by hand, and gently washed with tap water over 2 and 0.5 mm sieves. Root tips were collected per sample under a stereomicroscope for further enzymatic and molecular analyses (see Chapter 6). Bulk soil was sieved (2 mm), and stored at -20 ºC for further analyses. Remaining bulk soil was air-dried for physic-chemical analyses (see Chapter 5).

Molecular analyses, phylogenetic reconstruction, and phylogenetic metrics

Genomic DNA was extracted with the Invisorb®DNA Plant HTS 96 Kit/C kit (Invitek GmbH, Berlin, Germany) for root-tip samples (see details in Chapter 6), and with the PowerSoil kit (MoBio, Carlsbad, CA, USA) (500 mg of wet soil) for soil samples. The internal transcribed spacer region ITS-1 of the fungal nuclear ribosomal DNA was amplified with the primer pair ITS1F-ITS2 (Gardes and Bruns, 1993) adapted for Illumina-MiSeq (details in Chapter 6). Sequencing and bioinformatics analyses were conducted as previously described in Chapter 6. Fungal community phylogeny from 4166 fungal MOTUs was approximated with the software Phylomatic as implemented in Phylocom v.4.2 (Webb *et al.*, 2008) and BEAST v.1.5.4 (Drummond and Rambaut, 2007), and phylogenetic trees obtained as previously detailed in Chapters 3 and 4.

The phylogenetic structure of the fungal community was defined by two phylogeny-weighted metrics on the constructed trees: the Net Relatedness Index (NRI) and the PCPS index (see Chapters 3-4). The phylogenetic fuzzy-weighting method PCPS defines the phylogenetic community structure by calculating a matrix (matrix P) (Pillar and Duarte, 2010), where each MOTU has a value per sample that increases as the phylogenetic distance between neighbouring

MOTUs decreases. This matrix was calculated with the *PCPS* R-package (Debastiani *et al.*, 2015), and its dimensionality reduced by performing Principal Coordinate Analysis (PCoA) with Euclidean distances. Sample scores along the first principal component of the phylogenetic structure (PCPS1-Axe), which captures the deepest phylogenetic divergences among lineages (Duarte *et al.*, 2012), were extracted and used in further statistical analyses as a single variable describing the community phylogenetic structure (Pérez-Valera *et al.*, 2015). The contribution of each fungal phylum (mean ± SE) was calculated as the loadings of each taxon to the respective PCPS1 (see Chapters 3-4). To check for the reliability of our results regarding the phylogenetic tree used, we performed Pearson correlations between NRI and PCPS1 calculated with both the Phylocom and BEAST methods. The correlation values were 0.999, confirming the robustness of the analysis. For the sake of simplicity, we used a single tree for further analyses in each fungal community.

Enzymatic assays

The community functioning was evaluated by measuring activities of eight hydrolytic and oxidative exoenzymes secreted by fungi in the root tips and the bulk soil, by adapting the methods described by Mathieu *et al*. (2013) and Courty *et al*. (2005), as detailed in previous Chapters 2-4 and 6.

Genotyping of pine trees

In order to carry out a genetic characterization of the trees, genotyping of each tree (n = 15 for *P. pinaster* and n = 35 for *P. halepensis*) was performed with Illumina VeraCode® technology for a 384-plex single nucleotide polymorphism (SNP) Oligo Pool Assay (OPA) (251 successfully scored and polymorphic SNPs) enriched for well-known candidate genes for adaptive traits in forest trees (Budde *et al.*, 2014). This OPA includes polymorphic SNPs associated with climate variables in Mediterranean pines (*P. pinaster* and *P. halepensis*) (Grivet *et al.*, 2011) and a wide representation of functional candidate genes for biotic and abiotic stress responses, serotiny, physical and chemical wood properties, phenology and growth (Pot *et al.*, 2005; Eveno *et al.*, 2008; Grivet *et al.*, 2011; Lepoittevin *et al.*, 2012; Budde *et al.*, 2014).

Statistical analyses

Shapiro and Levene tests were performed to test respectively the normality and homocedasticity of all variables, which were log or square root transformed when needed.

In order to determine whether the fire regime had an effect on the phylogenetic structure of fungal communities in *P. pinaster* and *P. halepensis* forests, the phylogenetic indices NRI PCPS1 were analysed by General Linear Models (GLMs) with the fire regime (fix factor), and the site

(i.e., pine population) nested within the fire regime ($p<0.05$).

To determine if the tree genotype had an effect on the structure of their associated fungal communities, correlations between the dissimilarity matrices of fungal communities and the genetic matrix of trees (separately for each pine species) were performed with the function *mantel.rtest* (9999 permutations) in the ade4 R package. Tree genetic matrices derived from SNPs analysis were obtained with the function *dist.gene* in ape R package. The Bray-Curtis dissimilarity matrices of root-tip and soil fungal communities were used for these analyses, based on the abundance matrix of MOTUs previously normalized (i.e. variance stabilization) according to McMurdie and Holmes (2014) by using the DESeq R package (Anders and Huber, 2012). Additionally, dissimilarity matrices of the total community and of that of main phyla, i.e., Basidiomycetes and Ascomycetes, were calculated with the function *vegdist* in vegan R package. All these analyses were carried out with the R software v3.0.2 (R Core Team, 2014).

To investigate the effects of biotic (i.e., tree productivity), and abiotic (i.e., edaphic properties) factors, on the phylogenetic structure of root-tip and soil fungal communities associated with *P. pinaster* and *P. halepensis*, and the ecosystem functioning, we performed Structural Equation Modelling (SEMs) with AMOS v.20.0 software (IBM Corporation Software Group, Somers, NY), based on an aprioristic model (Figure S1) (see Chapters 2 and 4). Separated models per tree species and edaphic compartment (i.e., root-tips an soil) were done, and in each case the best-fitted model was chosen among the different causal models tested.

The diameter at breast height (DBH) was used as surrogate of tree productivity. Soil quality was predicted by Mid Infrared analysis: the second and third principal PCA components of the MIRs soil spectrum were used as proxies for the factor fire regime in *P. halepensis* and *P. pinaster* models, respectively (see Table 2 in Chapter 5 for details). Negative MIRs values were related with the effect of low fire recurrence and positive MIRs values with that of high fire recurrence. The phylodiversity index PCPS1 was used as indicator of the phylogenetic structure of the fungal community (see Chapter 4). Enzymatic processes representative of different nutrient cycles, i.e. glucosidase, cellobiohydrolase, xylosidase, glucuronidase, laccase for C; leucine and chitinase for N; acid phosphatase for P, were evaluated in separated models. It was hypothesized that productivity of trees and the fire-induced edaphic properties would influence the ecosystem functioning (i.e., enzymatic processes), directly and through modulating the phylogenetic structure of the associated fungal communities (Figure S1). Besides, a differential response of the fungal community settled in root tips *vs.* bulk soil (i.e., edaphic compartment) was expected.

RESULTS

The majority of fungal MOTUs were found in the soil compartment (Figure 1a). Most of the fungi identified in root tips were also found in soil (35.6% shared), while the remaining 62.3% was exclusively found in the bulk soil (Figure 1a). Both pine species shared 46.9% of MOTUs (40.6% in root tips; 45.2% in soil), and almost half of total fungi were found at both fire regime levels (48.8% for root tips; 52.2% for soil) (Figure 1b-c).

The top-10 fungal MOTUs highly diverged between edaphic compartments, pine species and fire recurrences (Table 1). In root tips of both pine species, ECM Basidiomycetes predominated, and in particular those within the genera *Tomentella* and *Amphinema*. By contrast, the 10 most abundant MOTUs in the bulk soil of *P. pinaster* and *P. halepensis* forests were saprotrophs Ascomycetes (e.g., *Archaeorhizomyces*), Basidiomycetes (e.g., *Cryptococcus*, *Geminibasidium*, *Sporidiobolales*), and Zygomycetes (e.g., *Mortierella*) (Table 1).

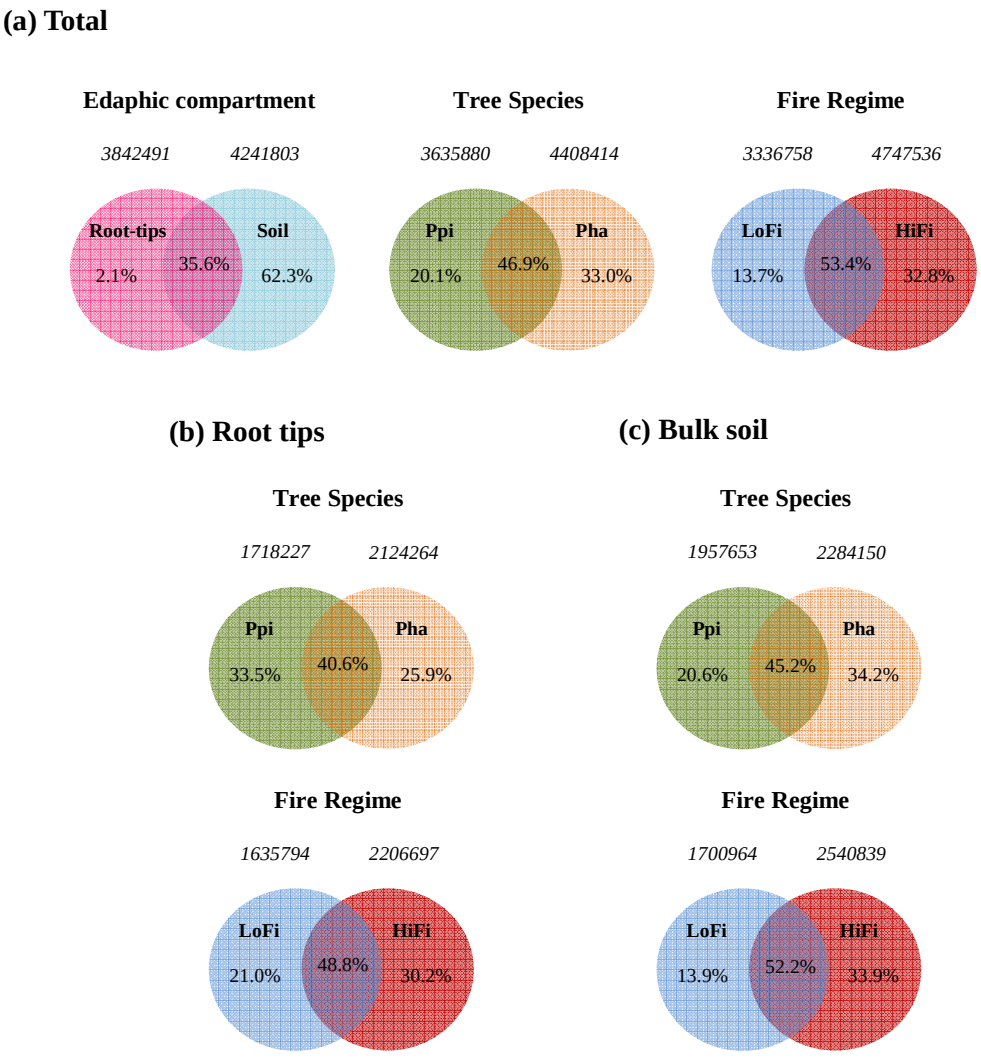

Figure 1 | No. sequences (*cursive*) and percentage of fungal MOTUs (a) total, (b) root tips and (c) bulk soil. Pine species: Ppi=*Pinus pinaster* and Pha=*Pinus halepensis*; and fire regime (LoFi = low fire recurrence and HiFi = high fire recurrence (serotinous populations).

Table 1 | The 10-most abundant fungal MOTUs found in the different edaphic compartments (a) root tips and (b) bulk soil of *Pinus pinaster* (Ppi) and *Pinus halepensis* (Pha) forests. No. of reads by tree species and fire regime, LoFi = low fire recurrence, HiFi = high fire recurrence. ECM = ectomycorrhizal; SAP = saprotrophic; END=endophytic; ? = unknown; ASC = Ascomycetes; BAS = Basidiomycetes ; ZYG = Zygomycetes.

(a) Root tips

			Tree Species		Fire Regime		Edaphic Co.
Pinus pinaster			**Ppi**	**Pha**	**LoFi**	**HiFi**	**Bulk Soil**
END	ASC	*Phialocephala fortinii*	91731	25914	50077	67568	6325
ECM	BAS	*Suillus bellinii*	89643	15198	34171	70670	27815
ECM	BAS	*Craterellus lutescens*	87541	10093	16	97618	51881
ECM	BAS	*Tylospora* sp.	80789	83	69703	11169	11515
ECM	BAS	*Rhizopogon* sp.	71158	11	12	71157	6036
ECM	BAS	*Amphinema* sp.	48695	125	27732	21088	1018
ECM	BAS	*Inocybe mixtilis*	35708	234	31893	4049	6822
ECM	BAS	*Russulaceae* sp.	35346	6180	20199	21327	6146
ECM	BAS	*Tomentella* sp.	33639	76899	61120	49418	4539
ECM	BAS	*Tomentella* sp.	31942	140486	45848	126580	12557
Pinus halepensis							
ECM	BAS	*Amphinema* sp.	27809	181050	81935	126924	22656
ECM	BAS	*Sebacinaceae*_A sp.	41	171441	41039	130443	17615
ECM	BAS	*Tomentella* sp.	31942	140486	45848	126580	12557
ECM	BAS	*Sebacinaceae*_ A sp.	21	82564	54754	27831	5381
ECM	BAS	*Amphinema* sp.	27962	79119	20092	86989	3208
ECM	BAS	*Tomentella* sp.	33639	76899	61120	49418	4539
ECM	BAS	*Tomentella* sp.	10604	54800	48466	16938	1305
PAT	ASC	*Ilyonectria macrodidyma*	918	51818	13719	39017	25101
SAP	BAS	*Sistotrema pistilliferum*	28119	47087	58527	16679	33861
ECM	BAS	*Tomentella coerulea*	24097	46119	28366	41850	2610

(b) Bulk soil

			Tree Species		Fire Regime		Edaphic Co.
Pinus pinaster			**Ppi**	**Pha**	**LoFi**	**HiFi**	**Root tips**
SAP	ZYG	*Umbelopsis* sp.	157431	8361	55002	110790	597
SAP	BAS	*Geminibasidium* sp.	85086	37306	50042	72350	89
SAP	ZYG	*Mortierella elongata*	74448	56793	54699	76542	2046
SAP	ASC	*Archaeorhizomyces* sp.	54408	227	8472	46163	1003
?	ASC	*Helotiales* sp.	49404	10230	19056	40578	218
?	BAS	*Sporidiobolales* sp.	48967	45847	37608	57206	483
ECM	BAS	*Craterellus lutescens*	47217	4664	186	51695	97634
SAP	BAS	*Cryptococcus podzolicus*	46402	32397	50145	28654	672
SAP	ZYG	*Umbelopsis* sp.	45185	26841	49703	22323	142
ECM	BAS	*Hydnellum auratile*	36847	58	20296	16609	16182
Pinus halepensis							
SAP	BAS	*Geminibasidium* sp.	15181	72135	24084	63232	40
SAP	ZYG	*Mortierella alpina*	2236	72084	46520	27800	192
ECM	BAS	*Tricholoma batschii*	22561	64233	2516	84278	56141
SAP	ZYG	*Mortierella elongata*	74448	56793	54699	76542	2046
SAP	ASC	*Archaeorhizomyces* sp.	5456	46927	28252	24131	84
?	BAS	*Sporidiobolales* sp.	48967	45847	37608	57206	483
SAP	BAS	*Cryptococcus aerius*	164	42725	21626	21263	63
SAP	BAS	*Geastrum fimbriatum*	17	40036	38277	1776	7
SAP	ASC	*Penicillium restrictum*	23432	39212	31143	31501	659
SAP	BAS	*Geminibasidium* sp.	85086	37306	50042	72350	89

Phylogenetic structure of fungal communities

The phylogenetic index NRI revealed divergent fungal structural patterns depending on the edaphic compartment and the pine species (Figure 2). The fungal community settled in root tips was clustered (positive NRI values) with respect to that in the bulk soil (i.e., over-dispersed) (Figure 2). The PCPS1 index, which captures deep phylogenetic differences among lineages, explained the 55.5 % and the 52.2 % of the total variance of fungal communities associated with the root tips of *P. pinaster* or *P. halepensis*, respectively. In the bulk soil, PCPS1 explained the 29.7 % of the variance in the case of *P. pinaster* and the 34.6 % for *P. halepensis*. A differential contribution of fungal phyla to the PCPS1 was observed depending on the edaphic compartment (Figure S2). In root tips of both *P. pinaster* and *P. halepensis*, Basidiomycota showed higher matrix P scores indicating a tendency of these fungi to co-exist with evolutionarily closer fungi, contrarily to Ascomycota and Zygomycota. However, in the bulk soil, the phylogenetic structure of the fungal community changed, and Basidiomycetes, which showed lower matrix P scores compared with that of the root-tips, tended to co-exist with evolutionarily distant fungi. For Ascomycetes and Zygomycetes the pattern was rather similar between edaphic compartments (Figure S2). The contribution of fungal phyla to the PCPS1 was relatively similar for both tree species (Figure S2).

Fire regime effect on the phylogenetic structure of fungal communities

The fire regime did not affect the phylogenetic structure of the fungal community associated with roots tips of *P. pinaster* or *P. halepensis* (Figure 3), although for *P. pinaster* the NRI index pointed to a marginal ($p = 0.058$) effect (i.e., over-dispersion of the fungal community in HiFi) (Table S3; Figure 2). Similar lack of response was observed in the bulk soil for *P. pinaster* (Figure 3; Table S3), but not for *P. halepensis*, where the fire regime significantly shaped the phylogenetic structure of the soil fungal community (Figure 3; Table S3). Specifically, the soil fungal community was less phylodiverse in *P. halepensis* forests under higher fire recurrence (Figure 2), where additionally Basidiomycetes were overrepresented and clustered (Figure 3; Table S3).

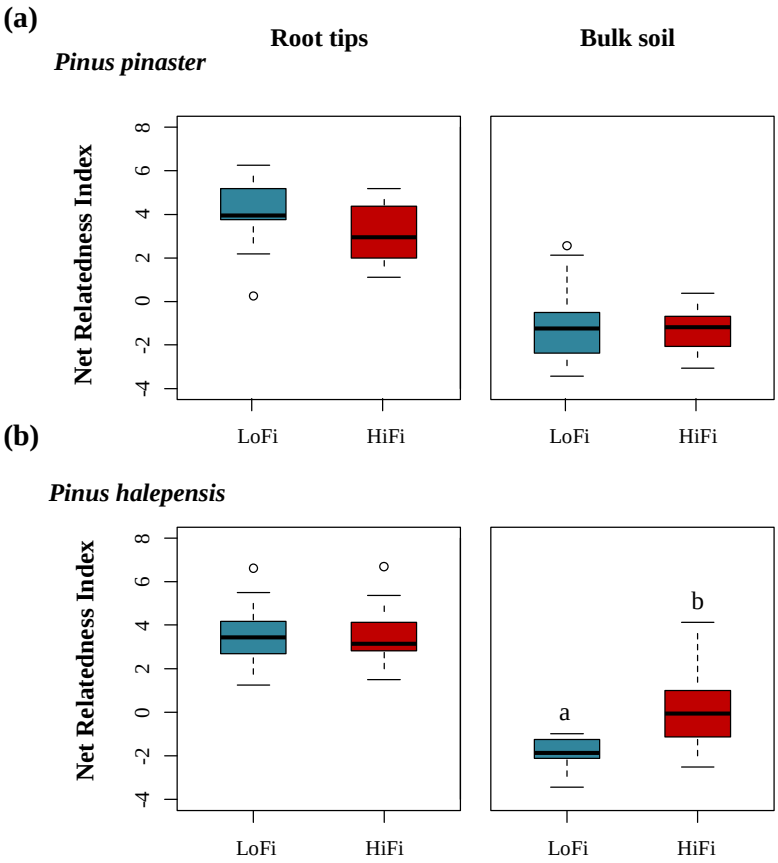

Figure 2 | Effect of the fire regime (blue= Low and red= High fire recurrence) on the Phylogenetic Net Relatedness Index (NRI) of fungal communities in root tips and bulk soil of (a) *P. pinaster* and (b) *P. halepensis.* Boxes represent the interquartile range (IQR) between first and third quartiles and the horizontal line inside is the median. Whiskers denote the lowest and highest values within 1.5 x IQR from the first and third quartiles, respectively. Within each graph, different letters denote significant differences among treatments ($p<0.05$).

Tree genotype effect on fungal community structure

The genetic structure of pines did not correlated with the phylogenetic assemblage of root tip fungal communities, but it did with those in the bulk soil (Table 2). The genetic matrix of *P. pinaster* significantly correlated with the phylogenetic assemblage of the total community and of that of Ascomycetes in the bulk soil (Table 2). For *P. halepensis*, it was the phylogenetic assemblage of Basidiomycota and ectomycorrhizal fungi, which correlated with the genetic structure of tress (Table 2).

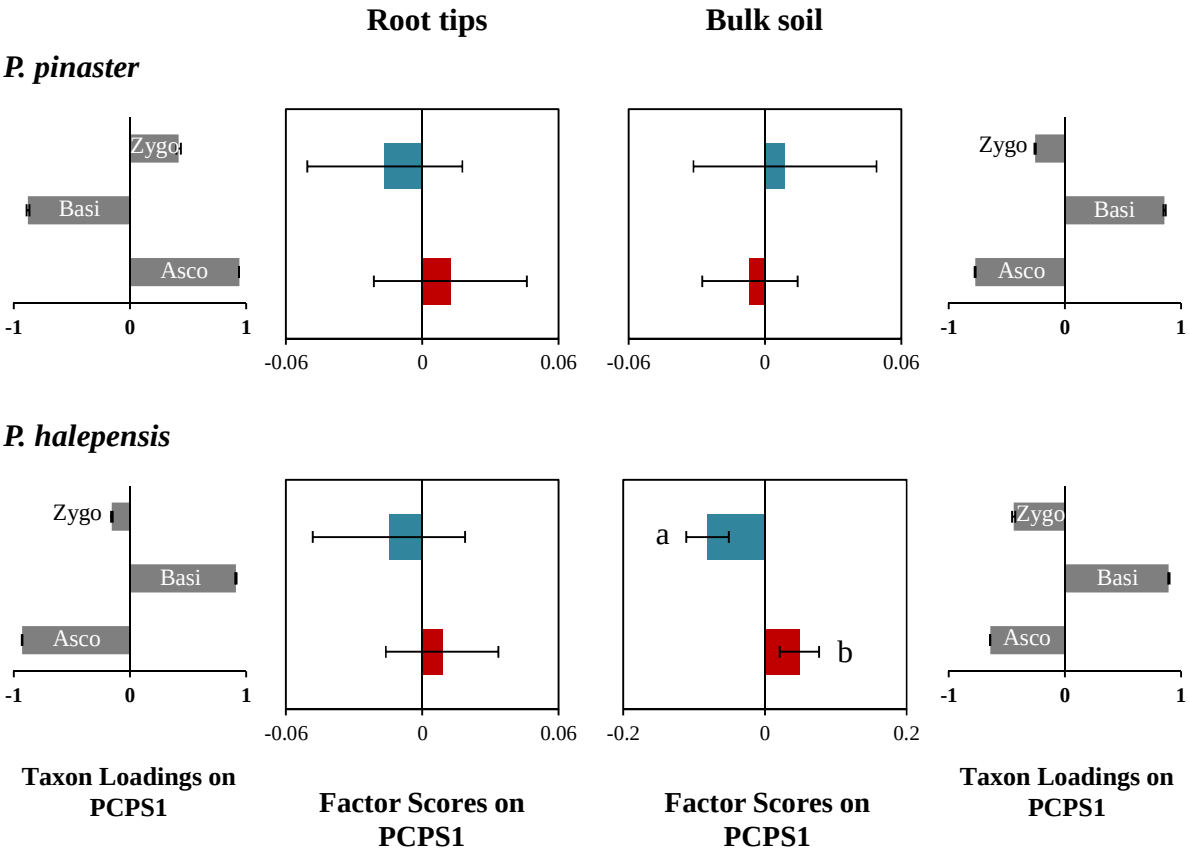

Figure 3 | Effect of the fire regime on the phylogenetic structure of fungal communities in root tips and bulk soil of *P. pinaster* and *P. halepensis*, analysed by the fuzzy-weighting method (PCPS). Grey bars show loadings (means ± SE) of each taxon on the first principal component (PCPS1) of the phylogenetic structure. In central graphics, colours indicate PCPS1 scores of different fire regimes (blue= Low and red= High fire recurrence). Within each graph, different letters denote significant differences among fire regime levels according to the Tukey test ($p<0.05$).

Relationships between fire regime, edaphic properties, fungal phylodiversity and ecosystem functioning

The best-fitted structural-equation models indicated different functional patterns for root-tip and bulk soil fungal communities in *P. pinaster* and *P. halepensis* forests (Figure 4).

In root tips of *P. pinaster*, the productivity of the trees directly and negatively influenced the phylogenetic structure of fungal communities, and the most productive trees were related to an overrepresentation of Basidiomycetes (Figure 4a). The fungal phylogenetic structure directly explained most enzymatic activities, and this effect was independent of the soil quality (i.e., fire regime); specifically, an overrepresentation of Basidiomycetes significantly explained higher N-cycle-enzymes and phosphatase activities, while a prevalence of Ascomycetes explained higher

levels of laccase. The hemicellulose-degrading activity was directly explained by the soil quality, i.e., lower under HiFi recurrence (Figure 4a). In the bulk soil of *P. pinaster* forests, neither the tree productivity nor the soil quality explained the phylogenetic structure of fungal communities, which in turn directly explained chitinase and hemicellulose-degrading activities (Figure 4a). Higher activities of these enzymes were further related to a preponderance of Basidiomycetes fungi (Figure 4a).

In the case of *P. halepensis* (Figure 4b), the phylogenetic structure of the root-tip fungal community was neither explained by the tree productivity nor the fire-induced soil quality. Nevertheless, an underrepresentation of Basidiomycetes explained low hemicellulose-degrading activity (Figure 4b). By contrast, in the bulk soil (Figure 4b), the fire effects on soil quality significantly shaped the phylogenetic structure of fungal communities, an overrepresentation of Basidiomycetes, which tended to co-exist with closer relatives, were observed under high fire recurrence. Moreover, these Basidiomycetes fungi explained high chitinase and phosphatase activities. On the other hand, the fire effects on soil quality directly explained higher laccase activity in soil, while leucine activity decreased (Figure 4b).

Table 2 | Mantel correlations between dissimilarity genetic matrices of *P. pinaster* and *P. halepensis* and dissimilarity matrices of total and by subgroups fungal communities for (a) root tips and (b) bulk soil. Significant correlations are indicated in bold ($p<0.05$); rM = Mantel correlation coefficient ; p-value from a test using 9999 permutations. BASI = Basidiomycetes; ASCO = Ascomycetes; ECM = ectomycorrhizal; SAP = saprotrophic.

		All MOTUs		**BASI**		**ASCO**		**ECM**		**SAP**	
		rM	p	rM	p	rM	p	rM	p	rM	p
Pinus pinaster	**Root tips**	-0.008	0.520	0.133	0.162	-0.113	0.760	0.083	0.210	-0.185	0.887
	Bulk soil	0.260	**0.023**	0.196	0.099	0.245	**0.024**	0.222	0.074	0.166	0.079
Pinus halepensis	**Root tips**	-0.021	0.547	0.151	0.102	-0.024	0.573	-0.001	0.509	-0.193	0.953
	Bulk soil	0.068	0.242	0.204	**0.044**	0.035	0.354	0.319	**0.011**	0.026	0.372

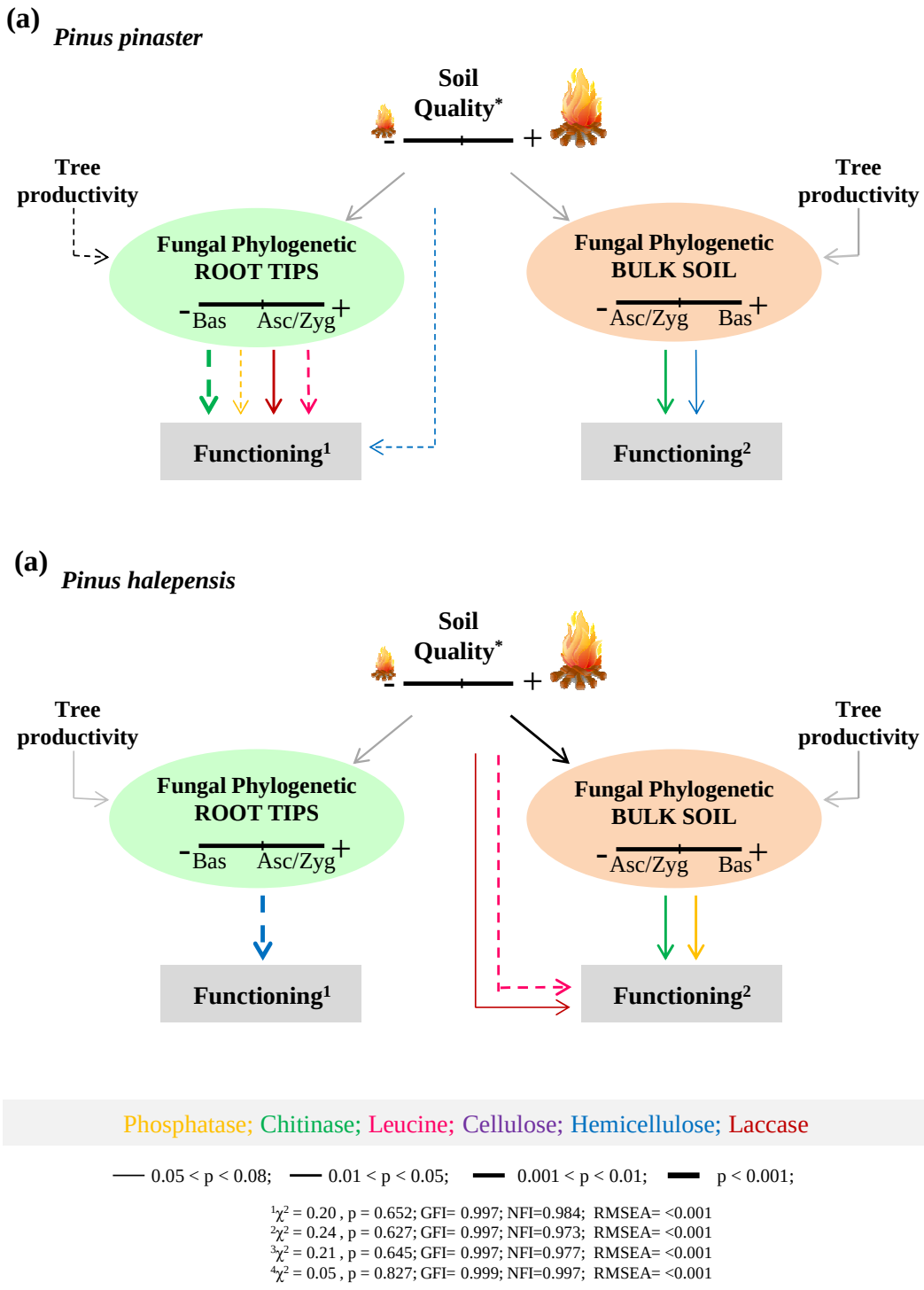

Figure 4 | Structural equation models representing causal relationships among the influence of the fire recurrence (PCA-Axis3 for *P. pinaster* PCA-Axis2 for *P. halepensis* of the Mid Infrared Soil analysis), tree productivity (diameter at breast height, DBH) and fungal phylogenetic structure (PCA-Axis1 of the PCPS matrix) associated with (a) *P. pinaster* and (b) *P. halepensis* on the ecosystem functioning. Independent models by edaphic compartment are shown for each pine species. Arrows indicate causal relationships: positive effects by solid lines, and negative effects by dashed lines. Different colours of arrows depict the hypothesized model for each enzymatic activity. Arrow widths are proportional to P values. DBH correlation with soil quality was taken into account to fit the model. Paths with coefficients non-significant different from 0 (p>0.08) are shown in grey. χ^2, p-value and fit statistics (NFI, GFI and RMSEA) of each model are also indicated.

DISCUSSION

Our results demonstrate that the genetics of Mediterranean pine populations is related to the phylogenetic structure of their associated fungal communities in soil, but surprisingly not in root-tips. Contrarily to that previously observed for α-diversity (Chapter 6), the fire regime does not affect the phylogenetic structure of root-tip fungal communities, but it prints a phylogenetic signal on those in the bulk soil, particularly in the case of *P. halepensis*. For this pine species, the high fire recurrence induces the phylogenetic clustering of soil fungal communities with the overrepresentation of Basidiomycetes. Our results demonstrate that variations in the phylogenetic structure of root-tip and soil fungal communities entail functional consequences related to the cycling of nutrients. In particular, the prevalence of Basidiomycetes in soils of high recurrently burned *P. halepensis* forests significantly increases the activities related to phosphorous and nitrogen mobilization. Overall our results reveal a divergent and compartmentalized structural and functional response of fungal communities to the fire regime in *P. pinaster* and *P. halepensis* forests.

Spatial distribution of belowground fungal communities

A high proportion of fungi were exclusively found in the bulk soil, and most of the fungal taxa identified in root tips were also found in soil. Our results agree with other authors that have pointed out to soil as a good estimate for fungal richness at a regional scale (Landeweert *et al.*, 2005; Coince *et al.*, 2013; Rincón *et al.*, 2015). However, species composition and the most abundant MOTUs highly diverged between the edaphic compartments. In root tips of both pine species, ectomycorrhizal Basidiomycetes predominated, while saprotrophic Basidiomycetes and Zygomycetes were dominant in the bulk soil. The small-scale spatial effects could be particularly important for ECM fungi due to their dual lifestyle, living as mycelium spreading in soil and in the root tips forming the symbiotic structures (Anderson *et al.*, 2014; Rincón *et al.*, 2015). In parallel, fungal communities were differently structured depending on the edaphic compartment, with that settled in root tips clustered (i.e., phylogenetically more homogeneous) with respect to that in the bulk soil (i.e., over-dispersed). The matrix P analyses indicated that shifts in fungal species within the phylum Basidiomycetes, highly represented in root-tips, together with a similar phylodiversity of Ascomycetes and Zygomycetes in root-tips and soil, probably governed these patterns together with selective host preferences (see Chapters 3,4,6). Root-tip fungal assemblages may reflect colonizing events partially stochastic, i.e., priority effects (Kennedy *et al.*, 2009), that would confer competitive advantage to the first arrivals for settling in a reduced space even under similar environmental conditions. Besides, other mechanisms, probably dominant in the bulk soil, such as competitive exclusion and limited shared resources would also restrict the number of taxa

coexisting within the same niche (Kennedy *et al.*, 2009; Roy-Bolduc *et al.*, 2016). Thus, different assembling forces could be unequally acting at each edaphic compartment.

Tree genetics and fire recurrence shape the phylogenetic structure of fungal communities

Tree genetics modulated the phylogenetic structure of their associated soil fungal communities, particularly for Basidiomycetes and ECM fungi in *P. halepensis* forests. According to Hart *et al.* (2005), our results could indicate that the environmental filter imposed by fire on fungal communities may be primarily driven by fire-induced changes in the tree community (i.e., serotiny, drought adaptation). Trees control microorganisms through the quantity and quality of resources they provide, the competition for nutrients, and mutualisms (i.e., mycorrhizas) (Wardle, 2002). Moreover, during the ecosystem development (e.g., progression and retrogression induced by long-history of recurrent burning), fungal community assembly processes are tightly coupled to the host identity (Martínez-García *et al.*, 2015) and to plant-soil feedbacks (Albornoz *et al.*, 2016). Probably different organic inputs of LoFi and HiFi tree genotypes may be recruiting different fungal consortia. Some authors have shown that fungal communities would be regulated not only by the carbohydrates and phytochemicals directly provided by trees (i.e., root-tips) (Rincon *et al.*, 2001; Chaparro *et al.*, 2013), but also by external delivery of complicated carbon polymers from litter (i.e., bulk soil) (Aučina *et al.*, 2007; Aponte *et al.*, 2010; Velmala *et al.*, 2013; Uroz *et al.*, 2016). In general, our results complement those previously obtained in Chapters 2 and 4, where the tree genotype is demonstrated a main component determining the fungal communities in Mediterranean pine forests.

Beyond the tree host influence, the fire regime clearly determined the phylogenetic structure of belowground fungal communities, although this impact was different depending on the edaphic compartment and the pine species. In HiFi *P. pinaster* populations, root-tip fungal communities were rather over-dispersed, and this was not observed in root tips of *P. halepensis*, were probably different assembling mechanisms operated. Due to the limitation of space, in root tips, priority effects or competitive exclusion may gain importance under any recurrent environmental disturbance such as fire, which can also affect the host fitness at the long-term. Genetic and/or phenotypic differences among tree species, e.g., root architecture and productivity (Abramoff and Finzi, 2015), as well as local adaptation of hosts and fungi would explain these results (Johnson *et al.*, 2010; Parladé *et al.*, 2011). Contrarily, in *P. pinaster* bulk soil no effect of fire was observed on the fungal phylogenetic structure, probably because the higher niche diversification in soil respect to root tips, and/or to compensated phylogenetic signals of main fungal guilds not allowing a clear pattern to emerge. In fact, in previous Chapter 4, fungal functional guilds, i.e., ectomycorrhizal and saprotrophs, in *P. pinaster* soils displayed the opposite

phylogenetic structural response, likely related with competence issues among phylogenetically closer clades in the respective fungal guilds (Fernandez and Kennedy, 2016). Contrary to *P. pinaster*, the fire regime significantly shaped the phylogenetic structure of the soil fungal community in *P. halepensis* forests. In this case, and following our hypothesis, the soil fungal community was phylogenetically clustered, and Basidiomycetes were overrepresented, which tended to co-exist with closer relatives. Many fire-ecology studies usually refer an overrepresentation of most resistant fungal taxa such as Ascomycetes after fire events (Torres and Honrubia, 1997; Holden *et al.*, 2013; Rincón *et al.*, 2014; Buscardo *et al.*, 2015; Reazin *et al.*, 2016). However, most of these studies are based on short-term fire effects, while at long-term, high fire recurrence can have more profound effects on plant and fungal communities and ecosystem development (e.g., pedogenesis) (Pausas, 2015; Albornoz *et al.*, 2016). Similar to our results, an increase of Basidiomycetes coupled with a decrease of Ascomycetes has been observed as time passed since fire, and it has been related with a higher proliferation of ectomycorrhizal fungi (i.e., mostly Basidiomycetes) (Holden *et al.*, 2013). In ecosystems historically subjected to frequent fires, the positive response of fungi would indicate a selection of the fire-tolerant ones over time (Dooley and Treseder, 2011; Rincón *et al.*, 2014; Buscardo *et al.*, 2015).

Interrelations among fire recurrence, soil quality, fungal phylodiversity and ecosystem functioning

Our results illustrated different mechanistic patterns for distinct tree species and edaphic compartments, in which specific fungal phylogenetic clades were identified associated with concrete ecosystem processes. The use of phylogenies has been previously pointed out to accurately describe the response of functional fungal traits to perturbations because phenotypic differences or similarities among species are founded in their evolutionary history, and hence fungal responses may be phylogenetically determined (Helmus *et al.*, 2010; Treseder and Lennon, 2015; Amend *et al.*, 2016).

Additionally, fire-induced changes on soil quality differentially affected the functional response of fungal communities, depending on the edaphic compartment and the pine species identity. In the case of *P. pinaster*, the phylogenetic structure of fungal community in root tips was influenced by the productivity of trees. Additionally, the phylogenetic structure of soil and root-tip fungal communities, i.e., an overrepresentation of Basidiomycetes, positively explained processes related to nitrogen and phosphorous cycles, and also with C turnover (positively or negatively depending on the edaphic compartment). In the case of *P. halepensis*, besides the different response depending on the edaphic compartment, through effects on the soil quality, the fire regime favored the overrepresentation of Basidiomycetes, and that explained also higher

phosphorous and nitrogen mobilization. We could thus predict that, inside a fungal community, Basidiomycetes can be filtered by the tree host identity and/or recurrent fire by their ability to mobilize limiting resources, as phosphorous and nitrogen. Moreover, our results seemed to reflect not only structural but also functional niche differentiation between edaphic compartments as previously indicated (Talbot *et al.*, 2013; Rincón *et al.*, 2015), since the phylogenetic structure of fungi settled in root-tips and soil explained different ecosystem functions. In fact, the rhizosphere influence generates particular micro-environmental characteristics, e.g. decrease of pH, compared with the bulk soil (Fageria and Stone, 2006), or production of phytochemicals (Walker *et al.*, 2003). Moreover, C:N:P stoichiometry and organic matter quantity/quality are considered among the major universal factors influencing soil enzymatic activities (Sinsabaugh *et al.*, 2008; Kivlin and Treseder, 2014; Courty *et al.*, 2016). Probably the differential organic inputs produced by each pine species, together with their contrasted edaphic environments, where bacteria may play important roles, are responsible for the functional divergences observed (Lynch and de Leij, 2001; Madritch *et al.*, 2006; Ojeda *et al.*, 2010; Courty *et al.*, 2011). In fact, it is possible that in *P. halepensis* forests, long-term high fire recurrence effects, inherent soil properties e.g., accumulation of recalcitrant organic forms coupled with basic pH, were high limiting for the fertility of soils with greater immobilization of nutrients, compared with acidic soils of *P. pinaster* forests (Fageria and Stone, 2006; Aznar *et al.*, 2016). Finally, it cannot be ruled out possible confounding effects of environmental covariation (e.g., climate) affecting fungal communities and the ecosystem functioning, as previously signaled in other field studies (Roy-Bolduc *et al.*, 2016).

Because little is known about how the assembly history of fungal communities impacts the ecosystem functioning (Fukami *et al.*, 2010), our results provide valuable information about structural and functional consequences of soil-plant-fungal feedbacks during the secondary succession and development of Mediterranean fire-prone ecosystems.

SUPPORTING INFORMATION

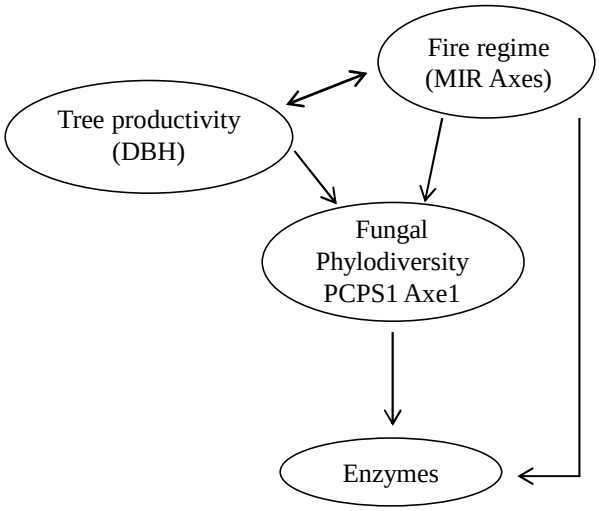

Figure S1 | Proposed path diagram representing hypothesized causal relationships among the influence of fire regime, tree productivity, phylogenetic structure of fungal communities and ecosystem functioning. Arrows depict causal relationships. Double sense arrow indicates correlations.

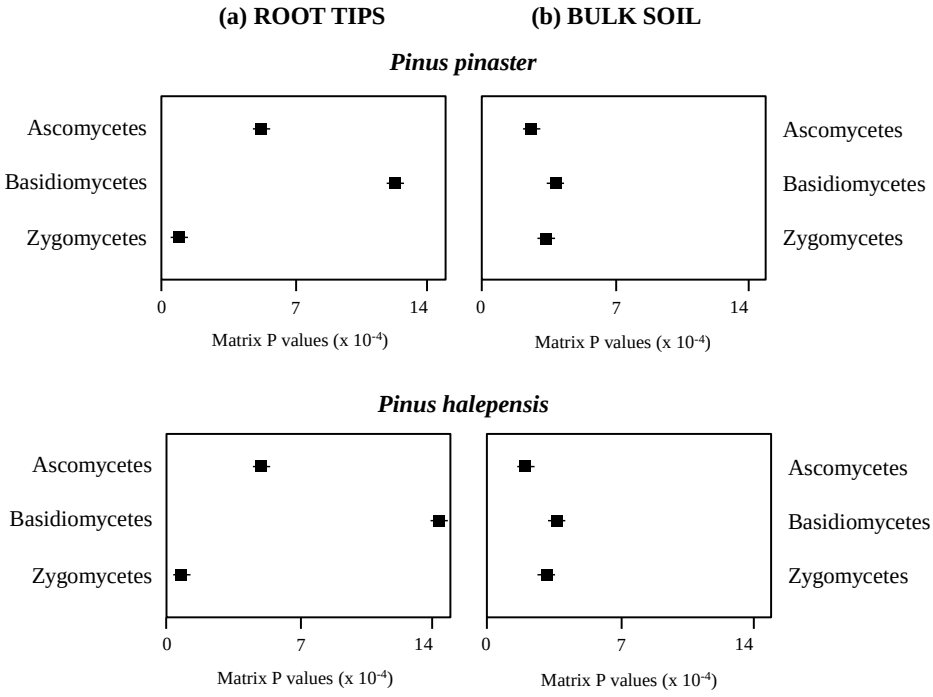

Figure S2 | Scores of main fungal phyla in matrix P, for (a) root-tips and (b) bulk soil in *P. pinaster* and *P. halepensis*. Values are means ± SD. In matrix P, each MOTU has a value per sample that increases as the phylogenetic distance between neighbouring MOTUs decreases.

Table S1 | Bibliographic references used to infer the relationships among distantly related taxa within the Fungal Kingdom and the age for major nodes in the phylogenetic "megatree", which was used to study the phylogenetic structure of fungal communities.

Kingdom	Phylum	Subphylum	Class/Subclass	Order	Family	
Fungi						
Ebersberger *et al.* (2012)	**Basidiomycota**	**Agaricomycotina**	**Homobasiodiomycetes**			
Hibbet *et al.* (2007)	Matheny *et al.* (2007)	Hibett (2006)	Binder *et al.* (2005)			
James *et al.* (2006)		Hibbet *et al.* (2014)	Bodensteiner *et al.* (2004)			
Larsson *et al.* (2007)			Larsson *et al.* (2004)			
Tedersoo *et al.* (2010)			**Agaricomycetes**	**Agaricales**	**Agaricaceae**	Vellinga (2004; 2011)
Tehler *et al.* (2003)			Floudas *et al.* (2012)	Matheny *et al.* (2006)	**Clavariaceae**	Dentinger and McLaughlin (2006)
			Hibbet *et al.* (2014)		**Entolomataceae**	Baroni and Matheny (2011)
					Hygrophoraceae	Lodge *et al.* (2014)
					Inocybaceae	Alvarado *et al.* (2010)
					Lyophillaceae	Sanchez-García *et al.* (2014)
					Psathyrellaceae	Nagy *et al.* (2011)
					Physalacriaceae	Henkel *et al* (2010)
					Pluteaceae	Justo *et al* (2011)
					Tricholomataceae	Sanchez-García *et al.* (2014)
				Atheliales		
				Kotiranta *et al.* (2011)		
				Larsson *et al.* (2004)		
				Boletales	**Boletaceae**	Wu *et al.* (2014)
					Gomphidiaceae	Miller (2003)
				Binder and Hibett (2006)		
				Wilson *et al.* (2012)		
				Cantharellales		
				Diederich *et al.* (2014)		
				Moncalvo *et al.* (2006)		
				Dacrymycetales		
				Kirschner *et al.* (2005)		
				Shirouzu *et al.* (2013)		
				Geastrales		
				Jeppson *et al.* (2013)		
				Hymenochaetales		
				Larsoon *et al.* (2006)		
				Polyporales		
				Binder *et al.* (2013)		
				Larsoon (2007)		
				Russulales		
				Miller *et al.* (2006)		
				Sebacinales		
				Oberwinkler *et al.* (2014)		
				Selosse *et al.* (2009)		
				Thelephorales	**Thelephoraceae**	Tedersoo *et al.* (2014)
				Larsson *et al.* (2004)		
				Auriculariales		
				Sotome *et al.* (2014)		
				Weib and Oberwinkler (2001)		
				Zhou *et al.* (2013)		
			Phallomycetidae	**Phallales**		
			Giachini *et al.* (2010)	Hosaka *et al.* (2006)		
				Gomphales		
				Hosaka *et al.* (2006)		
			Tremellomycetes	**Cystofilobasidiales**		
			Millanes *et al.* (2011)	Fell and Scorzetti (2004)		
			Wallemiomycetes			
			Hibbet *et al.* (2014)			
		Puccinomycotina	**Mycrobotriomycetes**			
		Libkind *et al.* (2011)	Sampaio *et al.* (2003)			
			Agaricostilbomycetes			
			Bauer *et al.* (2009)			

Continuation **Table S1**

Kingdom	Phylum	Subphylum	Class/Subclass	Order	Family
	Ascomycota		**Archaeorhizomycetes**		
	Schoch *et al.* (2009)		Menkis *et al.* (2014)		
			Dothideomycetes	**Dothideales**	
			Bohem *et al.* (2009)	Bills *et al.* (2012)	
			Schoch *et al.* (2009)	**Botryosphaeriales**	
				Slippers *et al.* (2013)	
				Capnodiales	**Capnodiaceae** Chomnunti *et al.* (2011)
				Crous *et al.* (2009)	
				Yang *et al.* (2014)	
				Pleosporales	**Pleosporaceae** Ariyawansa *et al.* (2015)
				Kodsueb *et al.* (2006)	**Venturiaceae** Crous *et al.* (2007); Machouart *et al.* (2014)
			Eurotiomycetes		**Trichocomaceae** Houbraken and Samson (2011)
			Chen *et al.* (2015)		**Verrucariaceae** Gueidan *et al.* (2007)
			Geiser *et al.* (2006)		
			Lecanoromycetes	**Lecanorales**	
			Miadlikowska *et al.* (2006; 2014)	Ekman *et al.* (2008)	
				Ostropales	
				Aptroot *et al.* (2014)	
				Teloschistales	
				Gaya *et al.* (2012)	
			Leotiomycetes		
			Cai *et al.* (2009)		
			Gernandt *et al.* (2001)		
			Hambleton and Sigler (2005)		
			Hambleton *et al.* (2005)		
			Wang *et al.* (2006a,b)		
			Geoglossomycetes		
			Wang *et al.* (2006b)		
			Orbiliomycetes		
			Wang *et al.* (2006b)		
			Pezizomycetes		**Pyronemataceae** Hansen *et al.* (2013); Sbissi *et al.* (2010)
			Perry *et al.* (2007)		
			Sordariomycetes	**Diaphortales**	
			Maharachchikumbura *et al.* (2015)	Castlebury *et al.* (2002)	
			Réblová *et al.* (2008)	**Sordariales**	**Lasiosphaeriaceae** Kruys *et al.* (2015)
			Summerbell *et al.* (2011)	Huhndorf *et al.* (2004)	**Chaetomiaceae** Morgenstern et al 2012
			Zhang *et al.* (2006)	**Xylariales**	
				Asgari and Zare (2011)	
				Jaklitsch and Voglmayr (2012)	
				Hypocreales	**Nectriaceae** Lombard *et al.* (2015)
				Chaverri *et al.* (2011)	**Hypocreaceae** Kullnig-Gradinger *et al.* (2002)
				Gräfenhan *et al.* (2011)	
				Johnson *et al.* (2009)	
	Glomeromycota				
	Kruger *et al.* (2012)				
	Redecker and Raad (2006)				
	Zygomycota			**Mucorales**	
	Chang *et al.* (2015)			Vitale *et al.* (2011)	
	White *et al.* (2006)			**Kickxellales**	
				Tretter *et al.* (2013)	
	Chytridiomycota			**Rhizophydiales**	
	James *et al.* (2006)			Letcher *et al.* (2008)	
				Lobulomycetales	
				Simmons *et al.* (2009)	
				Spizellomycetales	
				Wakefield *et al.* (2010)	

Node age datation
Amo de Paz *et al.* (2011)
Beimforde *et al.* (2014)
Berbee and Taylor (2010)
Chen *et al.* (2015)
Floudas (2012)
Hedges (2015)
Kohler *et al.* (2015)
Rouxel *et al.* (2011)

Table S2 | Effect of the fire regime on the phylogenetic structure of fungal communities associated with *P. pinaster* and *P. halepens*is in root-tips and bulk soil, measured as the Net Relatedness Index (NRI) and the fuzzy-weighting method (PCPS), and analysed by General Linear Models. df = degrees of freedom. F and p-value. Significant effects are indicated in bold (p<0.05).

		Root-tips		**Bulk Soil**	
Phylogenetic metrics	df	F	*p*	F	*p*
Pinus pinaster					
NRI	1	3.86	**0.058**	0.47	0.499
PCPS1	1	0.51	0.480	1.58	0.219
Pinus halepensis					
NRI	1	0.05	0.827	18.22	**0.000**
PCPS1	1	1.01	0.32	7.97	**0.008**

Chapter 8

A new promising molecular marker to study the functional diversity of fungal communities: the GLYCOSIDE HYDROLASE 63 gene

Photo: http://www.mycosphere.com.sg/index.htm

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Manuscript under review in *Molecular Ecology Resources*

INTRODUCTION

Fungi play main roles as decomposers, mutualists and pathogens in forest ecosystems, and are directly involved in biogeochemical nutrient cycling and tree productivity. Saprotrophic fungi are the main decomposer of organic matter generally located on the fresh litter, while in holartic regions, ectomycorrhizal (ECM) fungi dominate in organic and mineral soil horizons with an important role in nitrogen mobilization and carbon cycling (Lindahl *et al.* 2007; Clemmensen *et al.* 2013; Voříšková *et al.* 2014). Fungi secrete a wide set of extracellular proteins to decay biopolymers from dead organic matter and to interact with their partners and other organisms (Sinsabaugh, 2010; Talbot *et al.*, 2013; Baldrian, 2014; Pellegrin *et al.*, 2015).

Because forest resilience is highly dependent on the ecosystem services provided by fungi, a main goal in fungal community ecology is to predict the effects of disturbance (environmental change) on the ecosystem function (Talbot *et al.*, 2008; Koide *et al.*, 2014; Treseder and Lennon, 2015). Fungal secretomes are composed of several proteases, lipases, Carbohydrate-Active enZymes (CAZymes) or small-secreted proteins (SSP) (Alfaro *et al.*, 2014; Pellegrin *et al.*, 2015). Activities of these extracellular enzymes have been considered as traits to study the functional diversity of fungal communities (Cullings and Courty, 2009; Mathieu, Gelhaye, *et al.*, 2013; Talbot *et al.*, 2015), although these methods cannot indicate which fungi are responsible for these processes (Kellner & Vandenbol 2010). A complementary approach is the amplification of genes encoding for the corresponding proteins as indicators of putative fungal functions. With this perspective, different primers targeting potential fungal functional markers have been developed (e.g. gene fragments belonging to CAZyme families, such as laccases, cellobiohydrolases, chitinases, or pectinases) to describe jointly the taxonomic diversity and the role of soil fungal communities in carbon and nitrogen cycling (Lindahl and Taylor, 2004; Luis *et al.*, 2004; Bödeker *et al.*, 2009; Edwards *et al.*, 2011; Barbi *et al.*, 2014; Gacura *et al.*, 2016). Nevertheless, in a recent comparative genomics study, Kohler *et al.* (2015) have demonstrated pivotal differences among the diverse ecological fungal guilds (e.g. ECM fungi have a reduced set of genes encoding plant cell wall degrading enzymes compared to their ancestral wood decayers), which illustrates the difficulty to obtain an overview of the total fungal community owed to the lack of the universal distribution of these functional genes across the fungal kingdom. Moreover, using these markers as a measure of fungal abundance could be limiting because the multi-copy nature of the majority of these functional genes, as happens with the commonly used neutral internal transcribed spacer (ITS) marker, could provide blurred images of fungal diversity. As an example, the genome sequencing of *Aspergillus fumigatus* revealed a total of 529 CAZyme genes belonging to 115 families, made up of 1 to 37 different genes (Miao *et al.*, 2015). Therefore, functional diversity results are negatively affected by the multi-copy nature of these markers and the

existence of paralogues. Recently, Větrovský *et al.* (2016) proposed a fragment of a single-copy gene encoding the second largest sub-unit of RNA polymerase II (rpb2) as an alternative fungal marker. This gene can be very promising for fungal ecology studies, but it does not provide the link between the taxonomy and the functional potential of the fungal community.

In our study, we looked for a potential functional diversity marker with specific characteristics: highly reduced copy number, largely distributed across the fungal kingdom, able to be amplified with relatively well conserved primers, with barcoding and/or phylogenetic abilities, as well as targeting within secreted genes from the CAZyme family (i.e. universal function of carbon catabolism). Our main goal was to develop a new functional diagnostic molecular tool to monitor fungal communities in terms of diversity, structure, phylogeny and function, as a potential indicator of carbon cycling and secretome. We selected fungal genomes from the Mycocosm database (Grigoriev 2014) to identify potential candidates, and developed primers to amplify the single-copy Glycoside Hydrolase Family GH63 gene, encoding α-glucosidases, from a large collection of fungal genomic DNAs. The efficiency of this primer pair was compared with other published markers, and to evaluate its potential barcoding character, we compared the phylogenetic resolution of GH63 with one robust fungal phylogenetic marker, the Rpb1 gene.

MATERIAL AND METHODS

Fungal material

Sporocarps and ECM root tips were collected from forests around Champenoux, Nord-East France. The sporocarps were morphologically identified by Jean-Paul Maurice according to the new "French Reference of Mycology" (http://www.mycofrance.org), coordinated by Courtecuisse (2008). Both the ECM tips and a small piece of the inner part of the fruit-bodies were conserved at -20°C. Fungal pure cultures were obtained from the collections of INRA Nancy and ICA-CSIC Madrid (Table S1). After 3-4 weeks of growth, the mycelium was collected and immediately frozen in liquid nitrogen, before conservation at -20°C. A total of 125 fungal samples have been used in the present study: ectomycorrhizal and saprotrophic fungi belonging to 118 different species of Basidiomycota and 7 species of Ascomycota (Table S1).

Design of degenerated primers

Using the information of fungal genomes available in the MycoCosm database from JGI Genome Portal (http://genome.jgi-psf.org/programs/fungi/index.jsf), we selected different potential markers targeting within the CAZyme family, and based in the *a priori* criteria of single-copy gene and large distribution across fungal kingdom, GH9, GH63 and GH133 were the

selected genes. Nucleotidic sequences of these Glycoside Hydrolase Family genes were downloaded for different Basidiomycota species from MycoCosm database; the 22 different Basidiomycota species used in the case of GH63 are listed in the Table S2. A multiple alignment of these sequences was conducted with the MultAlin algorithm (Corpet, 1988). From sequences alignment, different conserved regions were selected as targets for primers design. Finally, among these potential candidates, we selected the primer pair that better amplified the gene GH63: GH63IF 5'- AGGGAYGARGGITTCCAYYT- 3' and GH63IR 5'- CGICGGAACCAITCRTARTG-3' (Table 1).

Table1 | Primers used in this study.

Gene family[1]	Primer name	Primer sequence (5'-3')	Target fungal group	Reference
GH63	GH63IF	AGGGAYGARGGITTCCAYYT	Basidiomycota	This study
	GH63IR	CGICGGAACCAITCRTARTG		
AA1 1	CuIF	CAYTGGCAYGGNTTYTTYCA	Basidiomycota	Luis *et al*. (2004)
	Cu2R	GRCTGTGGTACCAGAANGTNCC		
GH18	GH18 1b	ACIYTSGAYGCIATGAGYATG	Basidiomycota	Lindahl *et al*. (2004)
	GH18 2a	TCRTCICCRCCIGTRCTGAA		
GH7	FungcbhI-F	ACCAAYTGCTAYACIRGYAA	Basidiomycota/Ascomycota	Edwards *et al*. (2008)
	FungcbhI-R	GCYTCCCAIATRTCCATC		
AA2	Primer 1	GGIGGIGCIGAYGGITC	Basidiomycota	Bödeker *et al*.(2009)
	Primer 2	GGIGTIGARTCGAABGG		

[1]according to the CAZy database (http://www.cazy.org))

DNA extraction and identification of fungi

Genomic DNA from sporocarps, ECM tips and mycelium cultures was extracted using the DNeasy Plant Mini kit (Qiagen, Hilden, Germany) according to the manufacturer recommendations. Morphological identification of the fungal material was confirmed by the amplification of the universal DNA barcode for fungi (Schoch *et al.*, 2012), i.e. PCR amplifications of the ITS region were performed using the primers ITS1F/ITS4 (Gardes and Bruns, 1993) following the protocol of Buée *et al.* (2005).

PCR amplifications of GH63 and other functional markers

PCR amplifications with the GH63I primers were made in 20 µl reactions per sample containing 1 µl of genomic DNA, 2 µl of 10x polymerase buffer (Sigma-Aldrich), 0.7 µl of bovine serum albumin (16 mg ml^{-1}), 1 µl of dimethyl sulfoxide (DMSO), 0.4 µl of Nucleotides Mix (10 mM), 4 µl of 10 mM forward/reverse primers (GH63IF/GH63IR), and 0.1 µl of *Taq* DNA polymerase (5 U ml^{-1}) (Sigma-Aldrich). The optimized PCR conditions were: 94 ºC for 4 min, followed by 35 cycles of 94 ºC for 30 s, 55 ºC for 30 s and 72 ºC for 45 s, with a final step of 72 ºC for 5 min. Negative controls without DNA were included in all runs to detect possible

contaminations. The annealing temperature was optimized with PCR temperature gradients, the optimum temperature ranged from 50 to 55 °C. Moreover, on the same DNA templates, we compared the amplification rates of this new developed primers with those obtained with previous Basidiomycota primers targetting for other functional genes: laccase (Luis *et al.*, 2004), N-acetylhexosaminidase (Lindahl and Taylor, 2004), cellobiohydrolase I (Edwards *et al.*, 2008), and class II Peroxidase (Bödeker *et al.*, 2009). Supplemental PCR amplifications were accomplished using the primer pairs listed in Table 1, following the published protocols for each primer pair. When necessary, the efficiency of the amplification was increased adding 5% of DMSO to the total volume of PCR and/or changing the annealing temperatures. Success of PCR was assessed by ethidium bromide visualization after 1% agarose gel electrophoresis in 1x TBE buffer.

Sanger sequencing (Eurofins, Ebersberg, Germany) was carried out on all positive PCR products corresponding to amplified regions of ITS and GH63 genes. The consensus assembling of forward and reverse sequences was made using SeqMan 7v7.0.0, and manually edited. According to the best BLAST hit on NCBI and UNITE databases, the ITS sequences were taxonomically assigned (Table S1). The similarity of the putative GH63 protein fragments to known reference GH63 proteins was assessed with the algorithm BLAST in MycoCosm database (Table S3). ITS and GH63 sequences were deposited in the GenBank (Table S1).

Phylogenetic and secretome analysis

We downloaded the genomic resources of all GH63 genes from the 483 different fungal strains available in the MycoCosm database (Table S4). Indeed, we used the GH63 protein sequences to predict the presence of signal peptide cleavage sites in amino acid sequences (secretory pathway signal peptide), and to generate the phylogenetic trees approximated for maximum likelihood by the FastTree software (Price *et al.*, 2009). Moreover, a complete taxonomic identification and a putative life style were assigned to these strains following the classification of Tedersoo *et al.* (2014) (Table S4).

The phylogenetic trees corresponding to the marker Rpb1 (RNA polymerase II large subunit Rpb1) was also built. In order to estimate the potential barcoding and phylogenetic values of the GH63 gene, we compared this Rpb1 tree with the GH63 phylogenetic tree, for both *Basidiomycotina* and *Ascomycotina* clades, with the function *tanglegram* implemented in Dendroscope 3.2.10 (Huson and Scornavacca, 2012).

Because Glycoside Hydrolases of GH63 are exo-acting α-glucosidases, these proteins could have an important role on carbon cycle and should be potentially secreted, so we also tested this physiological character. Thereby, all GH63 protein sequences were analysed with five different methods: SignalP (Petersen *et al.*, 2011), WoLF PSORT (Horton *et al.*, 2006), TargetP

(Emanuelsson and Nielsen, 2000), TMHMM (Sonnhammer *et al.*, 1998) and ScanProsite (de Castro *et al.*, 2006), to predict the subcellular localization of these fungal proteins based on their N-terminal amino acid sequence.

RESULTS

PCR amplifications

Fungal species from a total of 73 different genera according to the similarity of NCBI/UNITE databases were studied (Table S1). Single bands of PCR products were obtained from 119 of 125 species amplified with the primer pair GH63I (Table S1). The success of amplification for the phylum Basidiomycota was 94 % for ECM and 99 % for saprotrophs (Table 2). As expected, a lower ratio of amplification was observed in Ascomycota (71 %), given the specificity of the designed primers for the Basidiomycota clade and the scarce number of Ascomycota species tested in our study. No amplification was obtained for the Ascomycota species *Chlorociboria aeruginosa* and *Helvella crispa* or for the Basidiomycota species *Cortinarius gratus*, *Hypholoma sublateritium*, *Lactarius decipiens* and *Russula fragilis*. Fragment sizes between 500-550 bp were obtained for a total of 85 species and between 420-480 bp for 31 species, whereas for the Ascomycota *Xylaria hypoxilon* and *Trichoderma* sp. a 400 bp fragment was got and for *Petriella setifera* the fragment was of 350 bp. The sequences corresponding to these fragments were compared with the MycoCosm protein database, and all the amplifications matched with GH63 fungal genes (Table S3).

Table 2 | Success of amplification (%) of different fungal markers tested on 125 fungal strains in the current study. ECM=ectomycorrhizal; SAP=saprotroph.

Phylum	Ecology	No. species	GH63I	CuIF/2R	GH18 1b/2a	FungcbhI	Peroxidase_II
Basidiomycota	ECM	49	**94**	55	33	12	**98**
	SAP	69	**99**	74	16	62	**97**
Ascomycota	SAP	7	71	29	0	57	100

Using the specific primer pair CuIF/2R for laccase, 55 % of ECM and 74 % of saprotrophic Basidiomycota amplified, while only 29 % of Ascomycota provided a positive yield (Table 2). Single fragments were variable in length (~150–1200 bp) for 14 different species. Double bands ranging between 150-350 bp were obtained also for 51 other fungal species. Finally, more than 2 fragments were amplified in 11 species.

The primer pair GH18 1b/2a, used for the amplification of a N-acetylhexosaminidase gene fragment, only gave positive results on Basidiomycota species and with very low success rates. Indeed, this functional marker was amplified for 33 % of ECM fungi and only for 16 % of saprotrophic fungi (Table 2). The length of amplicons varied between 400 and 650 bp. Two different bands were observed for *Pisolithus microcarpus* (400-500 bp) and *Pleurotus columbinus* (500-550 bp). This target was successfully amplified for all *Cortinarius* and *Xerocomus* species and for the majority of *Suillus* and *Hypholoma* species (Table S1).

Amplification rates of cellobiohydrolase gene with primers FungcbhI were very low when using ECM fungal DNA as template (12 %), while 62 % of saprotrophic Basidiomycota and 57 % of Ascomycota were successfully amplified with these same primers (Table 2). The most common fragment sizes ranged between 500-600 bp; the PCR fragments of ECM fungi were around 350, 450 and 1000 bp in length. Double amplifications were obtained for the species *Hymenopellis radicata*, *Pleurotus columbinus* and *Tricholoma acerbum* (Table S1).

Finally, the primers to screen the class II peroxidase gene were very efficient in terms of amplification rate. Indeed, with this primer pair, we amplified 48 species of ECM fungi (98 % of success) and 67 species of saprotrophs (97% of success) from the Basidiomycota clade. Despite these primers were specifically designed for this phylum, the rate of amplification of Ascomycota was 100 %. The number and sizes of PCR products were highly variable for the 111 species successfully amplified out of a total of 125 species (Table S1).

Table 3 | Distribution of GH63 gene within the different fungal phyla, obtained from the genomes of fungal species inventoried in the MycoCosm database (http://genome.jgi-psf.org/programs/fungi/index.jsf), and presence of GH63 (%) in the corresponding fungal species.

Phylum	No. Species / strains investigated	Presence (%)
Ascomycota	298	100
Basidiomycota	155	92
Chytridiomycota	5	60
Cryptomycota	1	100
Glomeromycota	1	100
Microsporidia	8	0
Zygomycota	15	100

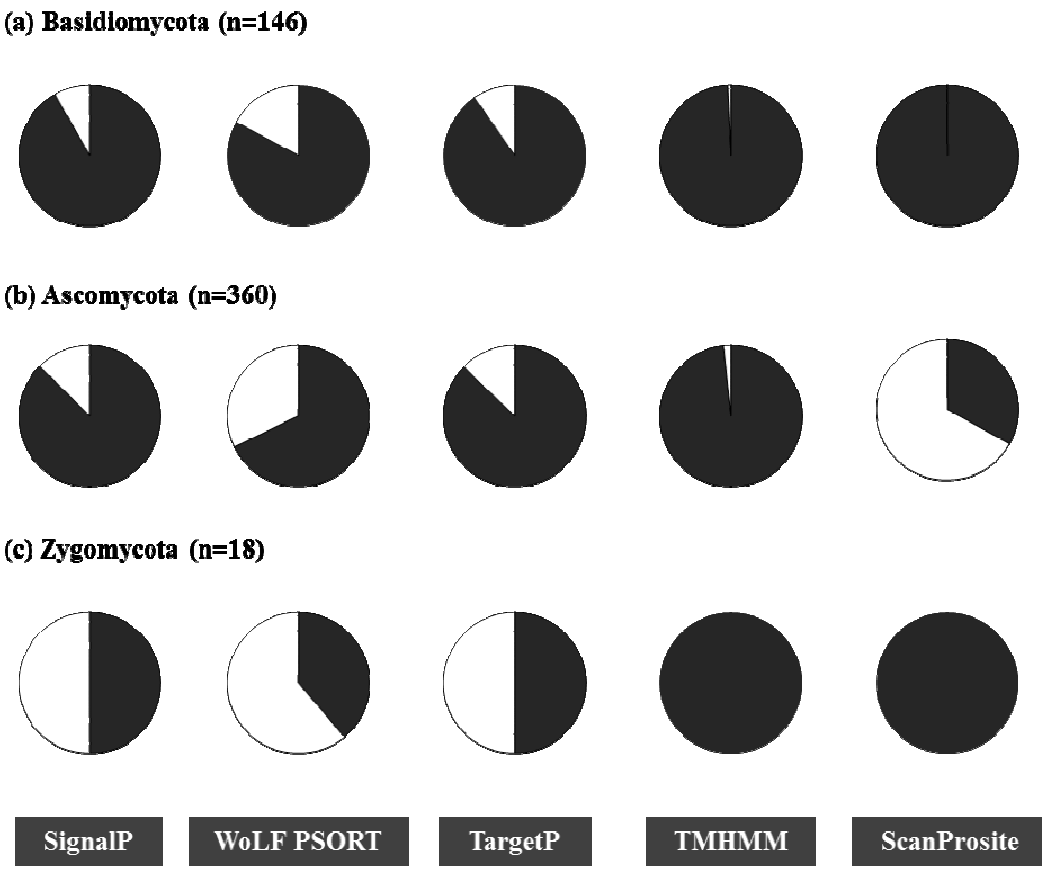


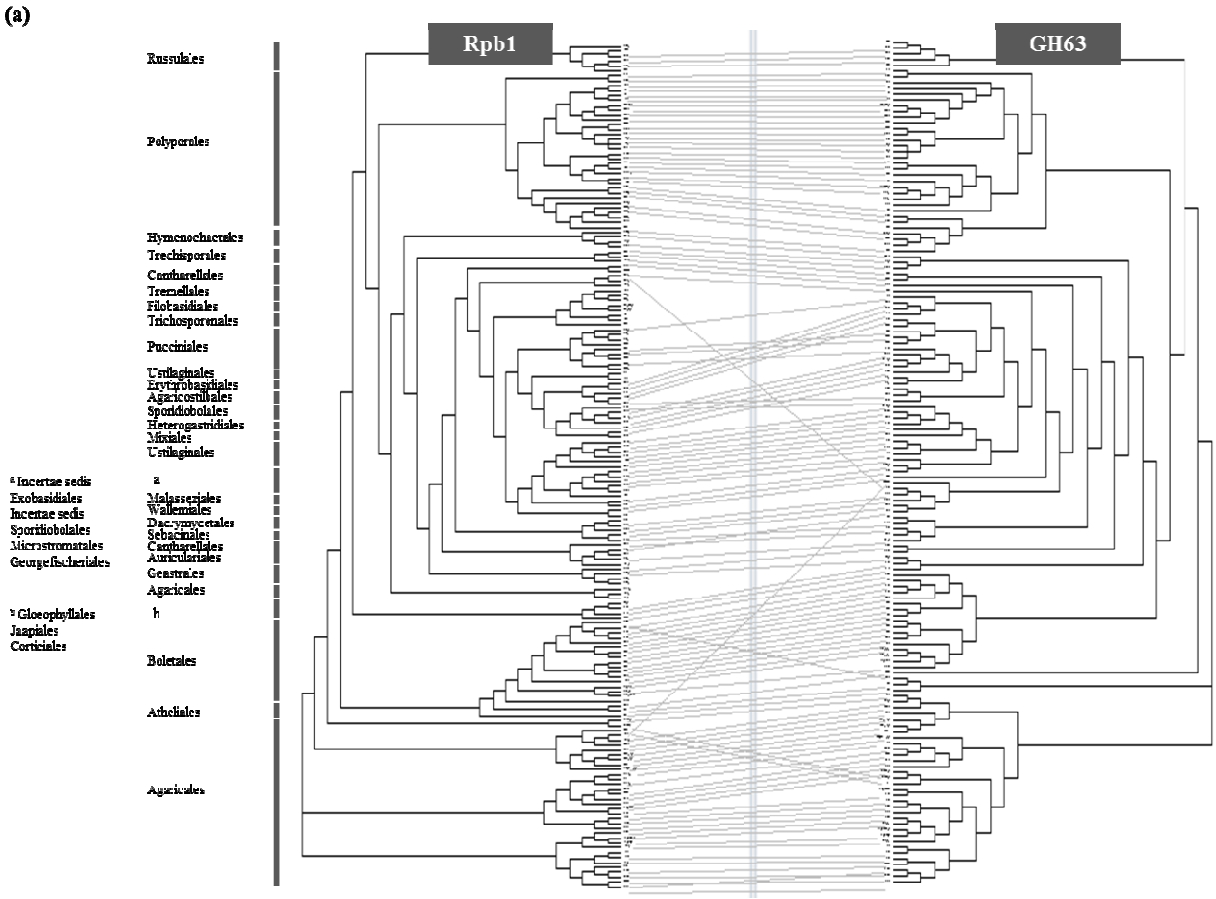
Figure 1 | Percentage of GH63 (Glycoside Hydrolase Family 63) secreted (black colour) and no secreted (white colour) protein in the different phylum (a) Basidiomycota (b) Ascomycota (c) Zygomycota tested by five different software.

GH63 I: marker of functional diversity, secretome and phylogeny

The GH63 gene was present in 461 strains out of a total of 483 strains whose genomes have been already sequenced (Table S4). By phylum, the GH63 gene was present in all the sequenced genomes of Ascomycota, Cryptomycota, Glomeromycota and Zygomycota (Table 3). No gene model corresponding to GH63 was found in the 11 remaining Basidiomycota genomes (*Cryptoccocus neoformans*, *Dioszegia cryoxeri*, *Jaminaea* sp., *Microstromatales* sp., *Moniliophthora perniciosa*, *Tremella mesenterica*, *Trichosporon chiarellii*, *Trichosporon oleaginosus*, *Tritirachium* sp., *Wallemia ichthyophaga* and *Wallemia sebi*), or in the two genomes of Chytridiomycota (*Orpinomyces* sp and *Piromyces* sp.). No strain belonging to the phylum Microsporidia had the GH63 gene (Tables 3; Table S4). In 398 strains, GH63 appeared as a single copy gene, constantly across all the ECM fungal species. Two copies of GH63 were found in the genomes of three Basidiomycota species (*Leucoagaricus gongylophorus*, *Naiadella fluitans* and *Postia placenta*), the Cryptomycota *Rozella allomycis*, the Zygomycota *Rhizopus oryzae* and in

the genome of 51 Ascomycota, all of them corresponding to pathogenic and saprotrophic species. The genome of the symbiotic Glomeromycota *Rhizophagus irregularis* also had two copies of GH63 (Table S4). Three GH63 copies were detected in the genome of *Ascocoryne sarcoides*, *Leptosphaeria maculans*, *Ophiobolus disseminans*, *Polyplosphaeria fusca* (Ascomycota) and *Backusella circina* (Zygomycota), and four copies in that of *Thozetella* sp. (Table S4). Therefore, GH63 was a single-gene in more than 86% of the fungal genomes.

Using five independent methods for sequences analysis, the fungal GH63 proteins were mainly predicted as secreted (Figure 1). In fact, the subcellular localization analysis of Basidiomycota GH63 showed a mean proportion of secreted proteins higher than 80%, and the ScanProsite method provided a rate of 100%. If a relatively high proportion of Ascomycota GH63 proteins was predicted as secreted by SignalP, WoLF PSORT, TargetP and TMHMM, this rate was lower with ScanProsite. For Zygomycota, 50% of GH63 proteins were predicted as secreted with the first three methods, whereas this proportion reached 100 % with the two last methods (Figure 1).



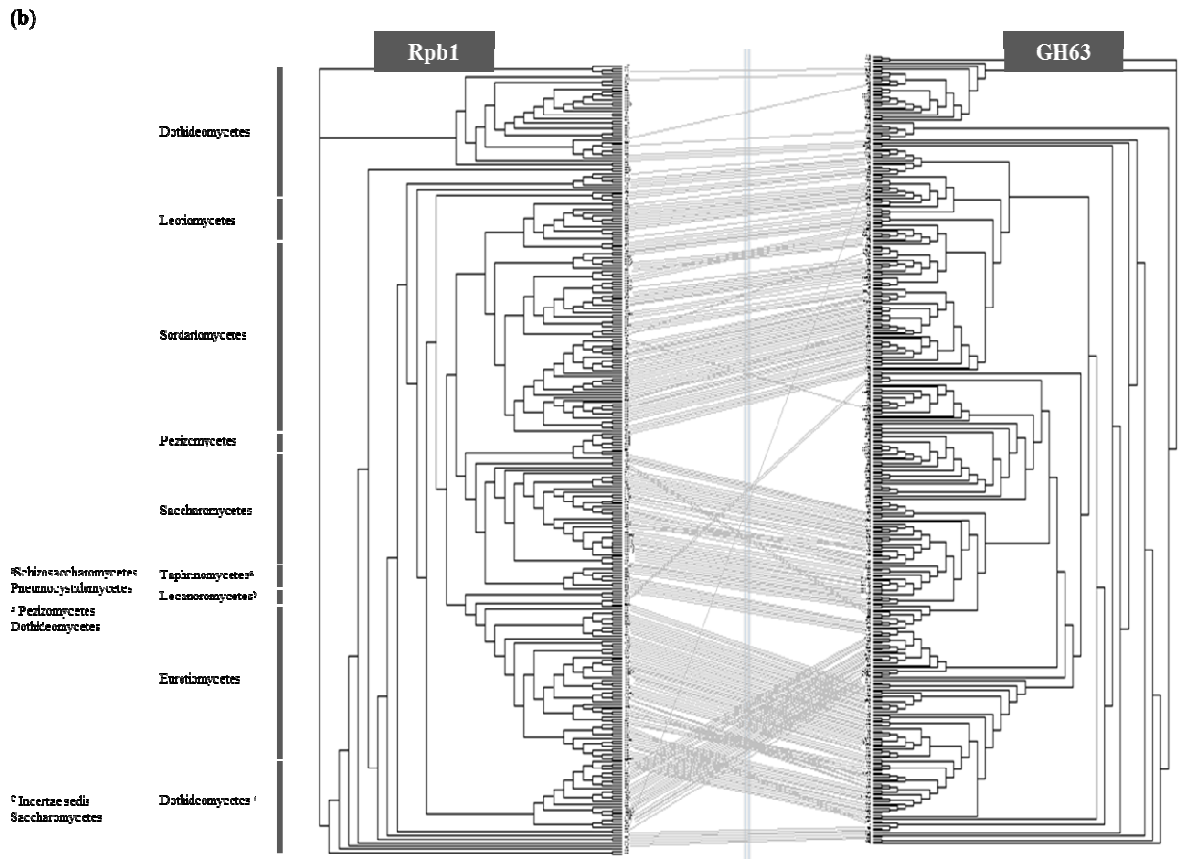


Figure 2 | Comparison of phylogenetic trees of Rpb1 (RNA polymerase II large subunit Rpb1) and GH63 (Glycoside Hydrolase Family 63) genes in (a) Basidiomycota and (b) Ascomycota phyla. Lines between both trees indicate the correspondence of taxa.

The phylogenetic character of the GH63 gene was demonstrated by the excellent analogy obtained when comparing the trees of the common phylogenetic fungal gene Rpb1 with that obtained with GH63 sequences (Figure 2). To make these Basidiomycota phylogenetic trees, 173 sequences of Rpb1 and 146 of GH63 were used from 154 and 143 fungal genomes respectively; that is because for Rpb1 19 duplications and zero gene loss events have occurred along the evolution, while in the case of GH63 three duplications of the gene have arisen. Taking into account the inherent differences resulting from the evolution of the gene trees and the possible incongruence respect to the species trees, both phylogenetic trees are topologically similar, as showed in Figure 2a. The most dissimilar topology between the two phylogenetic trees corresponded to the species *Cylindrobasidium torrendii* (Agaricales), *Clavulina* sp. (Cantharelales), *Punctularia strigosozonata* (Corticiales) and *Pleurotus ostreatus* (Agaricales), not well resolved in any locus. The same phylogenetic analysis was done with the clade Ascomycota, using 301 Rpb1 gene sequences from 294 fungal genomes and 358 GH63 sequences from 296 fungal genomes. The events that happened along the evolution of these genes were six

duplications for Rpb1, and 21 duplications and seven gene losses for GH63. In this case, the topology of the two trees was also consistent, but less than the one previously obtained for Basidiomycota. The apparent inconsistent species were *Eremomyces bilateralis* (Dothideomycetes), *Xylona heveae* (Xylonomycetes), *Trinosporium guianense* (Dothideomycetes), *Melanconium* sp. (Sordariomycetes), *Terfezia boudieri* (Pezizomycetes) and *Ascobolus immersus* (Pezizomycetes), as well as a complete clade of Dothideomycetes (Figure 2b).

DISCUSSION

Much work has been done in the past decade to develop approaches for biodiversity analysis using DNA barcoding and implemented databases (Nilsson et al. 2016). If DNA metabarcoding allows the massive identification of multiple species from a complex single bulk sample or from a single environmental sample (Taberlet *et al.*, 2012), the universality of primers is a challenge in molecular ecology for eukaryotic phyla (Hadziavdic *et al.* 2014). Many studies have investigated the taxonomic diversity of fungi, but there are less focused on functional diversity and functional genes (Uroz et al. 2016). Searching versatile taxonomic and functional fungal markers is currently crucial to study the ecology of fungal communities. In the present study, we have designed new primers for a functional gene and validated them in 73 different fungal genera: to our knowledge, this is the first report of general primers for fungal Glycoside-Hydrolase GH63 gene, encoding α-glucosidases for Basidiomycetes, providing a potential tool to link the community composition to fungal secretome and carbon metabolism. According to Stockinger *et al.* (2010) the primary criteria to select a fungal DNA barcode region are: universality, feasibility and species resolution. The current taxonomic reference marker ITS has been validated as the primary fungal barcode marker by the Consortium for the Barcode of Life (Schoch *et al.*, 2012), and we used this locus to confirm the taxonomic identification of 125 strains in our study. Nevertheless, the use of ITS markers has been criticised and the exploration of other molecular barcoding tools encouraged (Větrovský *et al.*, 2016), especially due to the lack of interspecific variability in some groups of fungi (Rehner and Buckley, 2005; Rojas *et al.*, 2010; Gazis *et al.*, 2011), and the intraspecific variation in the ITS region (Nilsson *et al.*, 2008; Kovács *et al.*, 2011). Moreover, unlike the single-gene targets, the multicopy nature of ITS in the fungal genomes can limit its value to estimate the relative abundance of fungal taxa (Větrovský *et al.*, 2016). Consequently recent studies have developed and applied non-coding rRNA region genes to investigate the functional diversity of fungal communities (Barbi *et al.*, 2016; Gacura *et al.*, 2016). But the strategy can be critical for these protein-coding gene families, better conserved at the amino-acid than the nucleotide level due to the degeneracy of the genetic code, and for this reason, the design of degenerate primers to amplifyall gene copies from all species is often an unachievable aim (Barbi *et al.*, 2014). A way to avoid this problem is the searching of single-copy genes for community analysis allowing the accurate measurement of fungal diversity and phylogenetic analyses. In this perspective, Aguileta *et al.* (2008) identified few genes, among the single-copy ones common in most fungal genomes that gave well-supported phylogenies. But their high polymorphism does not allow identifying short conserved regions for the development of primers, and for these practical reasons, these genes cannot be easily amplified and sequenced for a wide range of fungal species. In our study, the efficiency of GH63 primers was very high,

and single bands were recovered in all cases in line with genomics prediction (Table S3). Only two tested species (one *Trametes versicolor* and one *Russula ochroleuca*) provided very weak PCR amplicons, not sufficient for molecular identification by sequencing. For *Cortinarius amoenolens*, *Hypholoma sublateritium*, *Lactarius decipiens* and *Russula fragilis* no PCR products were obtained, probably due to the quality of the sporocarps rather than to the absence of the target gene since for example the gene was present in the genome of *H. sublateritium*. Neither for *Chlorociboria aeruginosa* or *Helvella crispa* any amplification was retrieved, pointing out to the low affinity of primers for Ascomycota. However, despite being specifically designed for Basidiomycota, our primers amplified 5 out of the 7 Ascomycota strains, indicating other different explanations, and more Ascomycota species should be tested and specific primers designed to monitor this relevant phylum.

The fungal genetic resources allowed us to show the broad distribution of GH63 gene across the phyla Ascomycota, Basidiomycota, Chytridiomycota and Zygomycota, validating the universality criterion for our selected fungal DNA barcode. Nevertheless, any GH63 copy was found within the eight sequenced genomes of microsporidia. Although microsporidia seem to be derived from an endoparasitic chytrid ancestor similar to *Rozella allomycis* (James *et al.*, 2006; Capella-Gutiérrez *et al.*, 2012), their affiliation to the fungal kingdom is still controversial (Lee et al 2010). That's why Schoch *et al.* (2012) excludes Microsporidia and Cryptomycota also in their study. Indeed, it has been recently suggested that novel descriptions should utilise the systematic affiliation 'Opisthokonta: Microsporidia' instead of 'Fungi: Microsporidia' (Vávra and Lukeš, 2013), and using new markers in phylogeny will improve our knowledge in Opisthokonta evolution.

The low amplification rates obtained with primers for cellobiohydrolase and N-acetylhexosaminidase genes underline the limitation of these markers to exhaustively describe fungal communities. This last gene, encoding for an exocellulase, has been recently used to compare potential cellulose decomposition in litter and soil horizons, and the difficulty to link community composition with the targeted function signalled (Baldrian *et al.*, 2012). In our study, we confirmed that cbhI gene mainly targets the saprotrophic community. For N-acetylhexosamines, Lindahl & Taylor (2004) developed degenerated primers and validated them with DNA of 28 fungal species, with 64% of amplification success. In our study, under the same conditions, this rate was relatively low i.e. 33% of successful amplification for ECM and only 16% for saprotrophic fungi. Because Lindahl & Taylor (2004) used only 2 saprotrophic strains and 26 ECM basidiomycetes, these results suggest a higher specificity of these primers for this last symbiotic group. Also in our study, the use of primers for laccase and class II peroxidase genes revealed strong multiband amplification patterns, compromising the quantitative

interpretations of diversity data. In fact, some amplified fragments for N-acetylhexosaminidase and class II peroxidase genes have been demonstrated to be unspecified (Lindahl and Taylor, 2004; Bödeker *et al.*, 2009). In the case of GH63 gene, we obtained 94% and 99% of successful amplification with the designed primers, for ECM and saprotrophic basidiomycetes respectively. These primers should be useful for fungal ecology studies in forest because Basidiomycota appear to dominate ECM fungi community in temperate and boreal forest soils (Courty et al. 2010).

Proteins belonging to the glycoside hydrolase family 63 are found in bacteria, archaea, and eukaryotes. Eukaryotic GH63 proteins are processing α-glucosidase I enzymes (mannosyl-oligosaccharide glucosidase (EC 3.2.1.106), which specifically hydrolyse the terminal glucose residues in the mannosyl-oligosaccharide Glc3Man9GlcNAc2 (Kilker *et al.*, 1981; Kurakata *et al.*, 2008). Other activities have been also demonstrated, corresponding to 3-α-D-glucan 3-glucohydrolase and α-D-glucoside glucohydrolase (Lombard *et al.*, 2014), as reported in the fungus *Rhizopus oryzae* (Battaglia *et al.*, 2011). Interestingly, Adams *et al.* (2011) proposed that a consortium of bacterial and fungal symbionts of *Sirex noctilio*, a woodwasp, has complementary functions for degrading woody substrates, with putative activities of microbial GH63 proteins. Recently, Zeiner *et al.* (2016) characterized and compared the protein composition of the secretomes of four cosmopolitan Mn(II)-oxidizing ascomycetes, showing that *Alternaria alternata* secretome exhibited the highest number of functionally unique identified CAZymes (7 proteins), including four GHs (1 each in GH63, GH65, GH67, and GH95 families). In our study, we confirmed that fungal GH63 proteins are largely secreted. Using five methods of sequences analysis, these proteins were predicted as secreted in more than 80% (100% with two methods) within the Basidiomycota division. Because of this subcellular localization and its possible roles in the endoplasmic reticulum (Helenius and Aebi, 2004; Barker and Rose, 2013), we proposed this molecular marker as potential indicator of the secretory machinery of fungal communities, which could be monitored under different environmental conditions in forest soil and rhizobiome or during organic matter degradation. The primers of fungal GH63 validated in our study provide a valuable tool in environmental metagenomics studies to link community composition to fungal functions.

At present, high-throughput metabarcoding approach is one of the most powerful tool to study fungal communities (Schmidt *et al.*, 2013). The primers developed in the present study were designed to amplify gene fragments compatible with the Illumina MiSeq sequencing technology applied to metagenomic and metatranscriptomic studies. An important limitation of these high-throughput techniques is the increasing amount of data generated and the lack of sequence information in public databases, making it difficult to assign functional gene sequences to fungal taxa (Barbi *et al.*, 2014). Indeed, if ITS rDNA, followed by b-tubulin (tub2), is a quantitatively

dominant marker in public databases, fungal GH63 genes are currently underrepresented. It seems therefore essential to integrate this marker in future studies, especially for annotations by Blast. But before we reach that stage, it is possible to make analyses of phylogeny, given its characteristics and phylogenetic attributes. Indeed, GH63 gene sequences seem to have also a strong added value for fungal phylogenetic studies, where the development of powerful phylogenetic markers is a key concern in this research area. Recent sequencing efforts resulted in multi-locus phylogenies, which have improved our understanding of fungal phylogeny (Lutzoni *et al.*, 2004; James *et al.*, 2006; Hibbett *et al.*, 2007; Aguileta *et al.*, 2008). In our study, comparative analyses of phylogenetic trees obtained with Rpb1 and GH63 genes revealed a high phylogenetic inference power. Because these genes are relatively well conserved, it would be possible to design new primers to amplify longer fragments enabling to include this marker in future multi loci phylogenetic analyses.

Finally, the primers developed in our study are suitable for amplification of GH63 genes in Basidiomycota fungi, but must be optimized or modified for Ascomycota. This molecular marker could serve as a new indicator of structure and diversity of fungal communities with barcoding and phylogenetic abilities, and to monitor these communities in terms of secretome indicator, at least for Basidiomycota, and with potential for other phyla.

SUPPORTING INFORMATION

Table S1 | List of the 125 fungal species used for the validation of the primer pairs targeting GH63 and three other functional markers. PCR product indicates the number of amplifications obtained with each marker in each fungal strain. S = sporocarp; M = mycelium pure culture; E = ectomycorrhizal root tip; ECM = ectomycorrhizal; SAP = saprotroph. *Strains belonging to the phylum Ascomycetes. Details about pure cultures from ICA-CSIC Madrid and INRA Nancy collections can be obtained from the authors of the current paper. [a] Luis *et al*. (2004); [b] Lindahl *et al*. (2004); [c] Edwards *et al*. (2008); [d] Bödeker *et al*. (2009). ** Full genome sequence avalaible on Mycocosm database (http://genome.jgi-psf.org/programs/fungi/index.jsf).

Species (base on morfology)	Species (NCBI/UNITE)	GenBank/UNITE matches	Identity	E-value	Sample type	Ecology	PCR product				
							GH63 I	CuIF/2R[a]	GH18 1b/2a[b]	Fungcbhl[c]	Peroxidase II[d]
Agaricus sylvaticus	*Agaricus cf. tenuivolvatus*	KJ548134	100	0.0	S	SAP	1	1	1	0	1
*Aleuria aurantiaca**	*Aleuria aurantia*	DQ491495	99	0.0	S	SAP	1	>2	0	0	>2
Amanita muscaria	*Amanita muscaria*	AB080983	99	0.0	S	ECM	1	2	0	0	>2
Amanita rubescens	*Amanita rubescens*	UDB000038	99	0.0	S	ECM	1	0	0	0	>2
Amanita spissa	*Amanita spissa*	EF493270	99	0.0	S	ECM	1	2	0	0	>2
Amphinema sp.	*Amphinema byssoides*	UDB017833	99	0.0	E	ECM	1	2	0	0	>2
Amylostereum areolatum	*Amylostereum areolatum*	KC865592	99	0.0	M	SAP	1	0	0	1	1
Bjerkandera adusta	*Bjerkandera adusta*	FJ608590	99	0.0	M	SAP	1	2	0	1	>2
Bjerkandera adusta	*Bjerkandera adusta*	JF439464	99	0.0	M	SAP	1	0	0	1	>2
Boletus reticulatus	*Boletus reticulatus*	KC422595	99	0.0	S	ECM	1	2	1	0	>2
Byssomerulius corium	*Byssomerulius corium*	UDB016376	99	0.0	M	SAP	1	0	0	1	1
Byssomerulius corium	*Byssomerulius corium*	UDB016376	99	0.0	S	SAP	1	0	0	1	2
Chalciporus piperatus	*Chalciporus piperatus*	UDB000427	99	0.0	S	ECM	1	2	0	0	>2
*Chlorociboria aeruginosa**	*Chlorociboria aeruginosa*	AY755358	99	0.0	S	SAP	0	0	0	1	2
Chlorophyllum rachodes	*Chlorophyllum olivieri*	AY081242	99	0.0	S	SAP	1	2	1	1	>2
Chondrostereum purpureum	*Chondrostereum purpureum*	GQ411519	99	0.0	M	SAP	1	2	0	1	>2
Clathrus archeri	*Clathrus archeri*	KJ702369	100	0.0	S	SAP	1	0	0	0	>2
Clavulina cristata	*Clavulina* sp.	JF519096	100	0.0	S	SAP	1	0	0	0	2
Clitopilus prunulus	*Clitopilus prunulus*	FJ770408	99	0.0	S	SAP	1	0	0	1	>2
Coniophora puteana	*Coniophora puteana*	JX501309	100	0.0	M	SAP	1	0	0	1	>2
Coprinellus micaceus	*Coprinellus micaceus*	KF156331	99	0.0	S	SAP	1	2	1	1	>2
Coprinopsis picacea	*Coprinopsis picacea*	JN943110	99	0.0	S	SAP	1	2	0	1	>2
Cortinarius amoenolens	*Cortinarius gratus*	KF732318	99	0.0	S	ECM	0	0	1	0	0
Cortinarius cyanobasilis	*Cortinarius pulchrifolius*	KJ705121	99	0.0	S	ECM	1	1	1	0	>2
Cortinarius largus	*Cortinarius squamosocephalus*	KF732432	100	0.0	S	ECM	1	0	1	0	1
Cortinarius sp.	*Cortinarius* sp.	JQ347077	96	0.0	S	ECM	1	0	1	0	2
Cortinarius sp.	*Cortinarius umbrinolens*	HQ604701	100	0.0	S	ECM	1	2	1	1	>2
Craterellus cornucopioides	*Craterellus cornucopioides*	JF907967	100	0.0	S	ECM	1	0	1	1	>2
Cystoderma sp.	*Cystoderma fallax*	UDB011404	99	0.0	S	SAP	1	2	0	1	>2
Daedalea quercina	*Daedalea quercina*	JQ700296	99	0.0	M	SAP	1	1	0	0	>2
Donkioporia expansa	*Donkioporia expansa*	HM536086	100	0.0	M	SAP	1	2	0	1	>2
Fibroporia vaillantii	*Fibroporia vaillantii*	KC585346	99	0.0	M	SAP	1	2	0	0	>2
Fibroporia vaillantii	*Fibroporia vaillantii*	JX501316	99	0.0	M	SAP	1	1	0	0	0
Fomes fomentarius	*Fomes fomentarius*	AY849305	99	0.0	M	SAP	1	2	0	1	1
Fomitopsis fraxinea	*Perenniporia fraxinea*	AM269792	99	0.0	M	SAP	1	1	0	1	2
Fomitopsis pinicola	*Fomitopsis pinicola*	JF340282	99	0.0	M	SAP	1	1	0	0	>2
Fomitopsis pinicola	*Fomitopsis pinicola*	JX501306	99	0.0	M	SAP	1	>2	0	0	>2
Fomitopsis sp.	*Perenniporia fraxinea*	AM269792	99	0.0	M	SAP	1	1	0	1	2
Ganoderma applanatum	*Ganoderma adspersum*	JN222418	100	0.0	M	SAP	1	2	0	1	>2
Ganoderma applanatum	*Ganoderma applanatum*	JX501311	100	0.0	M	SAP	1	2	0	1	>2
Gloeophyllum odoratum	*Gloeophyllum protractum*	HM536090	99	0.0	M	SAP	1	0	0	0	>2
Gomphidus glutinosus	*Gomphidus glutinosus*	UDB019745	99	0.0	S	ECM	1	0	0	0	2
Gymnopus fusipes	*Gymnopus fusipes*	FR686558	99	0.0	S	SAP	1	0	0	0	0
Hebeloma pseudofragilipes	*Hebeloma pseudofragilipes*	KT217558	99	0.0	S	ECM	1	0	1	0	2
*Helvella crispa**	*Helvella lacunosa*	AJ544211	96	0.0	S	SAP	0	0	0	0	>2
Heterobasidion annosum	*Heterobasidion abietinum*	KC492896	99	0.0	M	SAP	1	>2	0	0	2
Heterobasidion annosum	*Heterobasidion parviporum*	KC492952	100	0.0	M	SAP	1	>2	0	0	>2
Hydnum repandum	*Hydnum repandum*	AY817136	100	0.0	S	ECM	1	0	0	0	>2
Hymenopellis radicata	*Hymenopellis radicata*	GQ913392	99	0.0	S	SAP	1	2	0	2	2
Hypholoma fasciculare	*Hypholoma fasciculare*	FJ481034	99	0.0	S	SAP	1	0	1	1	>2
Hypholoma fasciculare	*Hypholoma fasciculare*	JX501298	100	0.0	M	SAP	1	1	0	1	>2
Hypholoma fasciculare	*Hypholoma fasciculare*	UDB019589	100	0.0	M	SAP	1	2	0	1	>2
Hypholoma sublateritium	*Hypholoma sublateritium*	AY818349	100	0.0	S	SAP	0	2	1	1	>2
Inocybe geophila var. lilacina	*Inocybe geophila var. lilacina*	UDB015341	99	0.0	S	ECM	1	>2	0	0	>2
Ischnoderma benzoinum	*Ischnoderma benzoinum*	JQ518274	99	0.0	M	SAP	1	2	0	1	>2
Kuehneromyces mutabilis	*Kuehneromyces mutabilis*	GU062262	99	0.0	S	SAP	1	0	1	1	>2
Laccaria amethystina	*Laccaria amethystina*	HM189773	99	0.0	S	ECM	1	0	0	0	>2
Laccaria bicolor	*Laccaria bicolor*	KC881087	99	0.0	M	ECM	1	2	0	1	>2
Laccaria laccata	*Laccaria bicolor*	JQ753771	100	0.0	M	ECM	1	>2	0	0	>2
Lactarius deterrimus	*Lactarius deterrimus*	UDB000297	100	0.0	S	ECM	1	2	0	0	>2

Continuation **Table 1**

Species (base on morfology)	Species (NCBI/UNITE)	GenBank/UNITE matches	Identity	E-value	Sample type	Ecology	PCR product				
							GH63 I	CuIF/2R[a]	GH18 1b/2a[b]	Fungcbhl[c]	Peroxidase II[d]
Lactarius sp.	*Lactarius chrysorrheus*	UDB015752	99	0.0	E	ECM	1	2	0	0	>2
Lactarius tabidus	*Lactarius decipiens*	KF432973	99	0.0	S	ECM	0	2	0	1	>2
Laetiporus sulphureus	*Laetiporus sulphureus*	AY835668	99	0.0	M	SAP	1	1	0	0	>2
Laetiporus sulphureus	*Laetiporus sulphureus*	JX501310	100	0.0	M	SAP	1	2	0	0	2
Leccinum aurantiacum	*Leccinum aurantiacum*	EF517299	98	0.0	S	ECM	1	2	0	0	>2
Leccinum crocipodium	*Leccinum crocipodium*	AF454590	99	0.0	S	ECM	1	0	0	0	>2
Lentinus cyathiformis	*Lentinus cyathiformis*	KC862286	98	0.0	M	SAP	1	2	0	0	>2
Lenzites betulinus	*Trametes betulina*	JN164983	99	0.0	M	SAP	1	1	0	1	2
Lepista nuda	*Lepista nuda*	FJ810156	99	0.0	S	SAP	1	2	0	1	>2
Marasmiellus ramealis	*Marasmiellus ramealis*	JF313670	99	0.0	S	SAP	1	2	0	0	2
Meripilus giganteus	*Meripilus giganteus*	FR686567	99	0.0	M	SAP	1	2	0	1	>2
Mycena inclinata	*Mycena inclinata*	UDB019606	99	0.0	S	SAP	1	2	0	1	2
Mycena rosella	*Mycena rosella*	JF908488	99	0.0	S	SAP	1	2	1	0	2
Paxillus ammoniavirescens	*Paxillus ammoniavirescens*	JN661713	99	0.0	M	ECM	1	0	0	0	>2
Paxillus involutus	*Paxillus involutus*	FR750011	100	0.0	M	ECM	1	0	0	0	>2
Perenniporia fraxinea	*Perenniporia fraxinea*	AM269793	99	0.0	M	SAP	1	1	0	1	2
*Petriella setifera**	*Petriella setifera*	JX501314	100	0.0	M	SAP	1	>2	0	0	>2
Phlebia radiata	*Phlebia radiata*	AY854087	99	0.0	M	SAP	1	0	0	1	>2
Phlebiopsis gigantea	*Phlebiopsis gigantea*	JF440577	99	0.0	M	SAP	1	0	0	0	>2
Piptoporus betulinus	*Piptoporus betulinus*	JQ700297	99	0.0	S	SAP	1	>2	0	0	>2
Piptoporus betulinus	*Piptoporus betulinus*	JX501312	99	0.0	M	SAP	1	2	0	0	>2
Piptoporus betulinus	*Piptoporus betulinus*	JX109856	98	0.0	M	SAP	1	>2	0	0	>2
Pisolithus microcarpus	*Pisolithus microcarpus*	JN847469	99	0.0	S	ECM	1	0	2	1	>2
Pleurotus columbinus	*Pleurotus columbinus*	FJ608593	99	0.0	M	SAP	1	2	2	2	>2
Pleurotus cornucopiae	*Pleurotus sapidus*	FJ810181	99	0.0	M	SAP	1	2	0	1	>2
Pleurotus ostreatus	*Pleurotus ostreatus*	UDB017904	100	0.0	M	SAP	1	2	0	1	2
Pluteus leoninus	*Pluteus leoninus*	KC147682	99	0.0	S	SAP	1	2	1	1	2
Postia placenta	*Postia placenta*	JX501308	100	0.0	M	SAP	1	0	0	0	>2
Postia placenta	*Postia placenta*	JX501308	100	0.0	M	SAP	1	0	0	0	>2
Rhizopogon roseolus	*Rhizopogon roseolus*	UDB001619	99	0.0	M	ECM	1	0	0	0	2
Rhizopogon sp.	*Rhizopogon verii*	FJ876174	99	0.0	M	ECM	1	2	0	0	>2
Rhodocollybia butyracea	*Rhodocollybia butyracea*	UDB017989	99	0.0	S	ECM	1	2	0	0	>2
Rickenella fibula	*Rickenella fibula*	UDB019493	99	0.0	S	SAP	1	2	0	1	>2
Russula atropurpurea	*Russula atropurpurea*	AF418618	99	0.0	S	ECM	1	2	0	0	2
Russula fragilis	*Russula fragilis*	UDB018436	82	6.00E-180	S	ECM	0	2	0	0	1
Russula nigricans	*Russula nigricans*	AM087260	91	2.00E-105	S	ECM	1	0	0	0	>2
Russula ochroleuca	*Russula ochroleuca*	HM189921	100	0.0	S	ECM	1	2	0	0	1
Russula risigalina	*Russula risigalina*	UDB011187	100	0.0	S	ECM	1	2	0	0	>2
Serpula himantioides	*Serpula himantioides*	AJ536025	99	0.0	M	SAP	1	0	0	0	>2
Serpula lacrymans	*Serpula lacrymans*	KC491847	99	0.0	M	SAP	1	>2	0	0	>2
Stropharia aeruginosa	*Stropharia aeruginosa*	UDB019703	99	0.0	S	SAP	1	2	1	1	1
Suillus bellinii	*Suillus bellinii*	HM347655	99	0.0	M	ECM	1	2	1	0	>2
Suillus collinitus	*Suillus collinitus*	AY935517	99	0.0	M	ECM	1	2	1	0	>2
Suillus collinitus	*Suillus collinitus*	DQ440567	99	0.0	M	ECM	1	0	0	0	1
Suillus luteus	*Suillus luteus*	KF937367	99	0.0	M	ECM	1	2	1	0	>2
Telephora terrestris	*Telephora terrestris*	JQ712012	99	0.0	M	ECM	1	0	0	0	>2
Tementella sp.	*Tomentella stuposa*	UDB002428	99	0.0	E	ECM	1	0	0	0	>2
Tomentella sp.	*Tomentella stuposa*	UDB002428	99	0.0	E	ECM	1	0	0	0	>2
Trametes gibbosa	*Trametes gibbosa*	KC176329	99	0.0	M	SAP	1	2	0	1	2
Trametes gibbosa	*Trametes gibbosa*	KC176329	99	0.0	M	SAP	1	2	0	1	2
Trametes trogii	*Trametes trogii*	HQ380781	99	0.0	M	SAP	1	>2	0	1	>2
Trametes versicolor	*Trametes versicolor*	KC176346	99	0.0	S	SAP	1	2	0	1	1
Trametes versicolor	*Trametes versicolor*	JX501313	100	0.0	M	SAP	1	1	0	1	>2
Trametes versicolor	*Trametes versicolor*	KC176325	98	0.0	M	SAP	1	1	0	1	>2
Trichoderma sp.*	*Trichoderma* sp.	KF225918	99	0.0	M	SAP	1	0	0	1	>2
Tricholoma saponaceum	*Tricholoma saponaceum*	DQ370440	98	0.0	S	ECM	1	2	0	0	>2
Tricholoma saponaceum	*Tricholoma saponaceum*	JQ888220	99	0.0	S	SAP	1	0	1	0	2
Tricholoma sp.	*Tricholoma acerbum*	AF377247	98	0.0	M	ECM	1	0	1	2	>2
Tricholoma sulphureum	*Tricholoma sulphureum*	AY462035	99	0.0	S	ECM	1	0	0	0	>2
Xerocomus badius	*Xerocomus badius*	HM190040	99	0.0	S	ECM	1	2	1	0	>2
Xerocomus chrysenteron	*Xerocomus chrysenteron*	HQ207691	99	0.0	S	ECM	1	1	1	0	1
Xerocomus ferrugineus	*Xerocomus subtomentosus*	DQ066370	97	0.0	S	ECM	1	0	1	0	2
*Xylaria hypoxilon**	*Xylaria arbuscula*	AY183369	99	0.0	S	SAP	1	0	0	1	>2
*Xylaria hypoxilon**	*Xylaria hypoxilon*	GU300096	99	0.0	S	SAP	1	0	0	1	>2

Table S2 | Fungal species used in this study for the design of degenerated primers. Protein ID indicates the accesion number for each sequence of GH63 gene in the MycoCosm database (http://genome.jgi-psf.org/programs/fungi/index.jsf).

Fungal species	Protein ID
Agaricus bisporus	217075
Amanita muscaria	185038
Boletus edulis	833958
Coprinopsis cinerea	3327
Cortinarius glaucopus	7235020
Formitiporia mediterranea	87056
Formitopsis pinicola	148297
Hebeloma cylindrosporum	438614
Laccaria amethystina	674156
Laccaria bicolor	670965
Paxillus involutus	88925
Paxillus rubicundulus	888937
Pisolithus microcarpus	628253
Pisolithus tinctorius	992809
Postia placenta	107259
Scleroderma citrinum	113630
Sebacina vermifera	327807
Suillus brevipes	857770
Suillus luteus	482026
Trametes versicolor	57800
Tricholoma matsusake	1303146
Tulasnella calospora	78158

Table S3 | Best BLAST of the recovered Glycoside Hydrolase GH63 gene fragments in the MycoCosm database.

Species (base on morfology)	Species (NCBI)	Identity	E-value	Best hit GH63 protein
Agaricus sylvaticus	*Agaricus cf. Tenuivolvatus*	89.1	2.66E-92	*Agaricus bisporus*
Aleuria aurantiaca	*Aleuria aurantia*	84.4	5.05-049	*Wilcoxina mikolae*
Amanita muscaria	*Amanita muscaria*	98.5	0-0	*Amanita muscaria*
Amanita rubescens	*Amanita rubescens*	89.9	5.38E-09	*Fibulorhizoctonia*
Amanita spissa	*Amanita spissa*	96.2	2.16E-14	*Amanita muscaria*
Amphinema sp.	*Amphinema byssoides*	86.3	7.61E-38	*Piloderma croceum*
Amylostereum areolatum	*Amylostereum areolatum*	86.6	1.29E-30	*Trametes cingulata*
Bjerkandera adusta	*Bjerkandera adusta*	97.4	0-0	*Bjerkandera adusta*
Bjerkandera adusta	*Bjerkandera adusta*	96.1	0-0	*Bjerkandera adusta*
Boletus reticulatus	*Boletus reticulatus*	95	0.00E+00	*Boletus edulis*
Byssomerulius corium	*Byssomerulius corium*	86.8	6.33E-26	*Phanerochaete chrysosporium*
Byssomerulius corium	*Byssomerulius corium*	86.8	1.13E-25	*Phanerochaete chrysosporium*
Chalciporus piperatus	*Chalciporus piperatus*	87.3	3.09E-55	*Paxillus involutus*
Chlorociboria aeruginosa	*Chlorociboria aeruginosa*			
Chlorophyllum rachodes	*Chlorophyllum olivieri*	86.6	5.73E-36	*Agaricus bisporus*
Chondrostereum purpureum	*Chondrostereum purpureum*	89.4	3.21E-18	*Phanerochaete chrysosporium*
Clathrus archeri	*Clathrus archeri*	91.8	5.26E-15	*Ramaria rubella*
Clavulina cristata	*Clavulina* sp.	94.3	9.91E-08	*Clavulina* sp.
Clitopilus prunulus	*Clitopilus prunulus*	88.1	5.94E-15	*Pleurotus ostreatus*
Coniophora puteana	*Coniophora puteana*	94.7	1.13E-176	*Coniophora puteana*
Coprinellus micaceus	*Coprinellus micaceus*	90.7	5.04E-142	*Coprinellus micaceus*
Coprinopsis picacea	*Coprinopsis picacea*	86	1.35E-36	*Coprinopsis cinerea*
Cortinarius amenolens	*Cortinarius gratus*			
Cortinarius cyanobasilis	*Cortinarius pulchrifolius*	89.9	3.32E-118	*Cortinarius glaucopus*
Cortinarius largus	*Cortinarius squamosocephalus*	91.5	2.52E-86	*Cortinarius glaucopus*
Cortinarius sp.	*Cortinarius* sp.	90.8	1.03E-78	*Cortinarius glaucopus*
Cortinarius sp.	*Cortinarius umbrinolens*	90.7	5.49E-88	*Cortinarius glaucopus*
Craterellus cornucopioides	*Craterellus cornucopioides*	90.3	5.89E-17	*Cantharellus anzutake*
Cystoderma sp.	*Cystoderma fallax*	87	1.00E-16	*Gymnopus luxurians*
Daedalea quercina	*Daedalea quercina*	99.4	0-0	*Daedalea quercina*
Donkioporia expansa	*Donkioporia expansa*	88.9	1.15E-77	*Dichomitus squalens*
Fibroporia vaillantii	*Fibroporia vaillantii*	88.2	1.13E-19	*Cerrena unicolor*
Fibroporia vaillantii	*Fibroporia vaillantii*	87.6	2.01E-17	*Cerrena unicolor*
Fomes fomentarius	*Fomes fomentarius*	85.2	2.92E-55	*Pycnoporus sanguineus*
Fomitopsis fraxinea	*Perenniporia fraxinea*	88.2	7.05E-48	*Polyporus brumalis*
Fomitopsis pinicola	*Fomitopsis pinicola*	98.4	0-0	*Fomitopsis pinicola*
Fomitopsis pinicola	*Fomitopsis pinicola*	98.5	0-0	*Fomitopsis pinicola*
Fomitopsis sp.	*Perenniporia fraxinea*	86	2.03E-49	*Pycnoporus coccineus*
Ganoderma applanatum	*Ganoderma adspersum*	91.2	1.45E-101	*Ganoderma* sp.
Ganoderma applanatum	*Ganoderma applanatum*	92.5	5.53E-115	*Ganoderma* sp.
Gloeophyllum odoratum	*Gloeophyllum protractum*	92.4	2.98E-28	*Laccaria amethystina*
Gomphidus glutinosus	*Gomphidus glutinosus*	87.1	2.21E-86	*Rhizopogon salebrosus*
Gymnopus fusipes	*Gymnopus fusipes*	91.9	1.91E-145	*Gymnopus androsaceus*
Hebeloma pseudofragilipes	*Hebeloma pseudofragilipes*	84.3	1.46E-26	*Hebeloma cilindrosporum*
Helvella crispa	*Helvella lacunosa*			
Heterobasidion annosum	*Heterobasidion abietinum*	94.7	8.83E-172	*Heterobasidium annosum*
Heterobasidion annosum	*Heterobasidion parviporum*	96	5.98E-168	*Heterobasidion annosum*
Hydnum repandum	*Hydnum repandum*	91.1	2.24E-65	*Hydnum rufescens*
Hymenopellis radicata	*Hymenopellis radicata*	90.5	8.51E-05	*Trichaptum abietinum*
Hypholoma fasciculare	*Hypholoma fasciculare*	90.2	3.16E-109	*Hypholoma sublateritium*
Hypholoma fasciculare	*Hypholoma fasciculare*	89.5	6.01E-113	*Hypholoma sublateritium*
Hypholoma fasciculare	*Hypholoma fasciculare*	89.7	5.54E-115	*Hypholoma sublateritium*
Hypholoma sublateritium	*Hypholoma sublateritium*			
Inocybe geophila var. lilacina	*Inocybe geophila var. lilacina*	87.6	3.7E-0.32	*Coprinopsis cinerea*
Ischnoderma benzoinum	*Ischnoderma benzoinum*	86.9	3.89E-26	*Stereum hirsutum*
Kuehneromyces mutabilis	*Kuehneromyces mutabilis*	88	5.95E-19	*Gymnopillus chrysopellus*

Continuation **Table S3**

Species (base on morfology)	Species (NCBI)	Identity	E-value	Best hit GH63 protein
Laccaria amethystina	*Laccaria amethystina*	99.1	0-0	*Laccaria amethystina*
Laccaria bicolor	*Laccaria bicolor*	99.1	0-0	*Laccaria bicolor*
Laccaria laccata	*Laccaria bicolor*	96.3	0.00E+00	*Laccaria bicolor*
Lactarius deterrimus	*Lactarius deterrimus*	89.9	1.19E-128	*Lactarius quietus*
Lactarius quietus	*Lactarius quietus*	99.5	0-0	*Lactarius quietus*
Lactarius sp.	*Lactarius chrysorrheus*	90.8	2.15E-137	*Lactarius quietus*
Lactarius tabidus	*Lactarius decipiens*			
Laetiporus sulphureus	*Laetiporus sulphureus*	94.6	0-0	*Laetiporus sulphureus*
Laetiporus sulphureus	*Laetiporus sulphureus*	94.7	2.24E-176	*Laetiporus sulphureus*
Leccinum aurantiacum	*Leccinum aurantiacum*	88	7.01E-83	*Xerocomus badius*
Leccinum crocipodium	*Leccinum crocipodium*	87.4	6.78E-78	*Xerocomus badius*
Lentinus cyathiformis	*Lentinus cyathiformis*	87.9	1.80E-17	*Jaapia argillacea*
Lenzites betulinus	*Lenzites betulinus*	87.4	1.84E-69	*Trametes versicolor*
Lepista nuda	*Lepista nuda*	99.7	0-0	*Lepista nuda*
Marasmiellus ramealis	*Marasmiellus ramealis*	87.6	2.50E-50	*Gymnopus androsaceus*
Meripilus giganteus	*Meripilus giganteus*	84.7	7.28E-23	*Phlebiopsis gigantea*
Mycena inclinata	*Mycena inclinata*	88.7	7.41E-154	*Panellus stipticus*
Mycena rosella	*Mycena rosella*	88.9	2.40E-39	*Panellus stipticus*
Paxillus ammoniavirescens	*Paxillus ammoniavirescens*	99.1	0-0	*Paxillus ammoniavirescens*
Paxillus involutus	*Paxillus involutus*	99.1	0-0	*Paxillus involutus*
Perenniporia fraxinea	*Perenniporia fraxinea*	86.2	1.40E-46	*Ganoderma* sp.
Petriella setifera	*Petriella setifera*	90.9	5.58E-17	*Ophiobolus disseminans*
Phlebia radiata	*Phlebia radiata*	86.8	5.16E-32	*Plebiopsis gigantea*
Phlebiopsis gigantea	*Phlebiopsis gigantea*	94.1	1.49E-169	*Phlebiopsis gigantea*
Piptoporus betulinus	*Piptoporus betulinus*	91	4.99E-120	*Formitopsis pinicola*
Piptoporus betulinus	*Piptoporus betulinus*	90.7	2.11E-121	*Formitopsis pinicola*
Piptoporus betulinus	*Piptoporus betulinus*	90.7	1.32E-121	*Fomitopsis pinicola*
Pisolithus microcarpus	*Pisolithus microcarpus*	97.9	0-0	*Pisolithus microcarpus*
Pleurotus columbinus	*Pleurotus columbinus*	95.9	0-0	*Pleurotus ostreatus*
Pleurotus cornucopiae	*Pleurotus sapidus*	94.9	0-0	*Pleurotus ostreatus*
Pleurotus ostreatus	*Pleurotus ostreatus*	95.7	0-0	*Pleurotus ostreatus*
Pluteus leoninus	*Pluteus leoninus*	87.4	4.73E-25	*Agaricus bisporus*
Postia placenta	*Postia placenta*	97.2	0-0	*Postia placenta*
Postia placenta	*Postia placenta*	96.5	0-0	*Postia placenta*
Rhizopogon roseolus	*Rhizopogon roseolus*	89.3	3.99E-116	*Rhizopogon salebrosus*
Rhizopogon sp.	*Rhizopogon verii*	88.3	5.01E-59	*Rhizopogon vinicolor*
Rhodocollybia butyracea	*Rhodocollybia butyracea*	87.5	2.10E-46	*Gymnopus androsaceus*
Rickenella fibula	*Rickenella fibula*	90.5	3.58E-14	*Heterobasidium annosum*
Russula atropurpurea	*Russula atropurpurea*	86	7.14E-34	*Lactarius quietus*
Russula fragilis	*Russula fragilis*			
Russula nigricans	*Russula nigricans*	88.5	7.49E-30	*Lactarius quietus*
Russula ochroleuca	*Russula ochroleuca*			
Russula risigalina	*Russula risigalina*	85.7	5.71E-39	*Lactarius quietus*
Serpula himantioides	*Serpula himantioides*	99.5	0-0	*Serpula lacrymans*
Serpula lacrymans	*Serpula lacrymans*	98.6	0-0	*Serpula lacrymans*
Stropharia aeruginosa	*Stropharia aeruginosa*	88.5	1.28E-27	*Hypholoma sublateritium*
Suillus bellinii	*Suillus bellinii*	92.9	8.25E-159	*Suillus luteus*
Suillus collinitus	*Suillus collinitus*	92.4	3.20E-151	*Suillus luteus*
Suillus collinitus	*Suillus collinitus*	92.2	9.98E-152	*Suillus luteus*
Suillus luteus	*Suillus luteus*	98.3	0-0	*Suillus luteus*
Telephora terrestris	*Telephora terrestris*	88.9	6.80E-60	*Telephora ganbajun*
Tomentella sp.	*Tomentella stuposa*	84.8	1.92E-46	*Thelephora ganbajun*
Tomentella sp.	*Tomentella stuposa*	84.8	4.15E-44	*Thelephora ganbajun*
Trametes gibbosa	*Trametes gibbosa*	88.4	5.42E-71	*Trametes cingulata*
Trametes gibbosa	*Trametes gibbosa*	88.6	3.55E-70	*Trametes cingulata*
Trametes trogii	*Trametes trogii*	86.3	2.66E-45	*Trametes ljubarskyi*
Trametes versicolor	*Trametes versicolor*			
Trametes versicolor	*Trametes versicolor*	94.8	0-0	*Trametes versicolor*
Trametes versicolor	*Trametes versicolor*	92.9	5.69E-141	*Trametes versicolor*
Trichoderma sp.	*Trichoderma* sp.	95	1.54E-169	*Trichoderma harzianum*
Tricholoma saponaceum	*Tricholoma saponaceum*	88.3	2.05E-61	*Tricholoma matsutake*
Tricholoma saponaceum	*Tricholoma saponaceum*	88.9	5.74E-75	*Tricholoma matsutake*
Tricholoma sp.	*Tricholoma acerbum*	85.4	1.67E-32	*Tricholoma matsutake*
Tricholoma sulphureum	*Tricholoma sulphureum*	88.8	1.81E-52	*Tricholoma matsutake*
Xerocomus badius	*Xerocomus badius*	99.3	0-0	*Xerocomus badius*
Xerocomus chrysenteron	*Xerocomus chrysenterom*	91.5	2.44E-1150	*Xerocomus badius*
Xerocomus ferrugineus	*Xerocomus subtomentosus*	86.2	5.24E-56	*Xerocomus badius*
Xylaria hypoxilon	*Xylaria arbuscula*	100	1.02E-02	*Colleototrichum falcatum*
Xylaria hypoxilon	*Xylaria hypoxilon*	100	8.46E-03	*Colleototrichum falcatum*

Table S4 | Sequenced fungal species whose genome was available at date of may 2015 in MycoCosm database (http://genome.jgi-psf.org/programs/fungi/index.jsf). The table shows the taxonomy, the JGI nomenclature, the number of copies of GH63 gene and the ecology of these especies.

Kingdom	Phylum	Class	Order	Family	Genus	Species	JGI nomenclature	Number of copies	Ecology
Fungi	Ascomycota	Dothideomycetes	Pleosporales	Dacampiaceae	*Aaosphaeria*	*Aaosphaeria arxii*	Aaoar1	1	Saprotroph
Fungi	Ascomycota	Dothideomycetes	Capnodiales	Teratosphaeriaceae	*Acidomyces*	*Acidomyces richmondensis*	AciriA	1	Pathogen
Fungi	Ascomycota	Dothideomycetes	Capnodiales	Teratosphaeriaceae	*Acidomyces*	*Acidomyces richmondensis*	AciriB	1	Pathogen
Fungi	Ascomycota	Sordariomycetes	Hypocreales	Incertae sedis	*Acremonium*	*Acremonium alcalophilum*	Acral2	1	Saprotroph
Fungi	Ascomycota	Sordariomycetes	Hypocreales	Incertae sedis	*Acremonium*	*Acremonium strictum*	Acrst1	1	Saprotroph
Fungi	Basidiomycota	Agaricostilbomycetes	Agaricostilbales	Agaricostilbaceae	*Agaricostilbum*	*Agaricostilbum hyphaenes*	Agahy1	1	Saprotroph
Fungi	Basidiomycota	Agaricomycetes	Agaricales	Agaricaceae	*Agaricus*	*Agaricus bisporus*	AgabiA	1	Saprotroph
Fungi	Basidiomycota	Agaricomycetes	Agaricales	Agaricaceae	*Agaricus*	*Agaricus bisporus*	AgabiB	1	Saprotroph
Fungi	Ascomycota	Dothideomycetes	Pleosporales	Pleosporaceae	*Alternaria*	*Alternaria alternata*	Altal1	2	Pathogen
Fungi	Ascomycota	Dothideomycetes	Pleosporales	Pleosporaceae	*Alternaria*	*Alternaria brassicicola*	Altbr1	2	Pathogen
Fungi	Basidiomycota	Agaricomycetes	Agaricales	Amanitaceae	*Amanita*	*Amanita muscaria*	Amamu1	1	Ectomycorrhizal
Fungi	Basidiomycota	Agaricomycetes	Agaricales	Amanitaceae	*Amanita*	*Amanita thiersii*	Amath1	1	Saprotroph
Fungi	Ascomycota	Dothideomycetes	Pleosporales	Amniculicolaceae	*Amniculicola*	*Amniculicola lignicola*	Amnli1	2	Saprotroph
Fungi	Ascomycota	Eurotiomycetes	Incertae sedis	Amorphothecaceae	*Amorphotheca*	*Amorphotheca resinae*	Amore1	1	Saprotroph
Fungi	Ascomycota	Sordariomycetes	Xylariales	Diatrypaceae	*Anthostoma*	*Anthostoma avocetta*	Antav1	2	Pathogen
Fungi	Microsporidia	Microsporea	Microsporida	Nosematidae	*Antonospora*	*Antonospora locustae*	Antlo1	0	Biotroph
Fungi	Basidiomycota	Agaricomycetes	Polyporales	Fomitopsidaceae	*Antrodia*	*Antrodia sinuosa*	Antsi1	1	Saprotroph
Fungi	Ascomycota	Sordariomycetes	Xylariales	Apiosporaceae	*Apiospora*	*Apiospora montagnei*	Apimo1	2	Saprotroph
Fungi	Ascomycota	Dothideomycetes	Botryosphaeriales	Botryosphaeriaceae	*Aplosporella*	*Aplosporella prunicola*	Aplpr1	2	Pathogen
Fungi	Basidiomycota	Agaricomycetes	Auriculariales	Polyporaceae	*Aporpium*	*Aporpium caryae*	Elmca1	1	
Fungi	Basidiomycota	Agaricomycetes	Agaricales	Physalacriaceae	*Armillaria*	*Armillaria gallica*	Armga1	1	Saprotroph
Fungi	Basidiomycota	Agaricomycetes	Agaricales	Physalacriaceae	*Armillaria*	*Armillaria mellea*	Armme1	1	Saprotroph
Fungi	Ascomycota	Orbiliomycetes	Orbiliales	Orbiliaceae	*Arthrobotrys*	*Arthrobotrys oligospora*	Artol1	1	Saprotroph
Fungi	Ascomycota	Eurotiomycetes	Onygenales	Arthrodermataceae	*Arthroderma*	*Arthroderma benhamiae*	Artbe1	1	Saprotroph
Fungi	Basidiomycota	Agaricomycetes	Polyporales	Polyporaceae	*Artolenzites*	*Artolenzites elegans*	Artel1	1	Saprotroph
Fungi	Ascomycota	Pezizomycetes	Pezizales	Ascobolaceae	*Ascobolus*	*Ascobolus immersus*	Ascim1	1	Saprotroph
Fungi	Ascomycota	Leotiomycetes	Helotiales	Helotiaceae	*Ascocoryne*	*Ascocoryne sarcoides*	Ascsa1	3	Saprotroph
Fungi	Ascomycota	Pezizomycetes	Pezizales	Ascodesmidaceae	*Ascodesmis*	*Ascodesmis nigricans*	Ascni1	1	Saprotroph
Fungi	Ascomycota	Saccharomycetes	Saccharomycetales	Ascoideaceae	*Ascoidea*	*Ascoidea rubescens*	Ascru1	1	Saprotroph
Fungi	Ascomycota	Saccharomycetes	Saccharomycetales	Eremotheciaceae	*Eremothecium*	*Ashbya gossypii*	AshgoA	1	Pathogen
Fungi	Ascomycota	Eurotiomycetes	Eurotiales	Trichocomaceae	*Aspergillus*	*Aspergillus acidus*	Aspfo1	1	Saprotroph
Fungi	Ascomycota	Eurotiomycetes	Eurotiales	Trichocomaceae	*Aspergillus*	*Aspergillus aculeatus*	Aspac1	1	Saprotroph
Fungi	Ascomycota	Eurotiomycetes	Eurotiales	Trichocomaceae	*Aspergillus*	*Aspergillus brasiliensis*	Aspbr1	1	Saprotroph
Fungi	Ascomycota	Eurotiomycetes	Eurotiales	Trichocomaceae	*Aspergillus*	*Aspergillus campestris*	Aspcam	1	Saprotroph
Fungi	Ascomycota	Eurotiomycetes	Eurotiales	Trichocomaceae	*Aspergillus*	*Aspergillus carbonarius*	Aspca3	1	Saprotroph
Fungi	Ascomycota	Eurotiomycetes	Eurotiales	Trichocomaceae	*Aspergillus*	*Aspergillus clavatus*	Aspcl1	1	Saprotroph
Fungi	Ascomycota	Eurotiomycetes	Eurotiales	Trichocomaceae	*Aspergillus*	*Aspergillus flavus*	Aspfl1	1	Saprotroph
Fungi	Ascomycota	Eurotiomycetes	Eurotiales	Trichocomaceae	*Aspergillus*	*Aspergillus fumigatus*	Aspfu1	1	Saprotroph
Fungi	Ascomycota	Eurotiomycetes	Eurotiales	Trichocomaceae	*Aspergillus*	*Aspergillus fumigatus*	Aspfu_	1	Saprotroph
Fungi	Ascomycota	Eurotiomycetes	Eurotiales	Trichocomaceae	*Aspergillus*	*Aspergillus glaucus*	Aspgl1	1	Saprotroph
Fungi	Ascomycota	Eurotiomycetes	Eurotiales	Trichocomaceae	*Aspergillus*	*Aspergillus kawachii*	Aspka1	1	Saprotroph
Fungi	Ascomycota	Eurotiomycetes	Eurotiales	Trichocomaceae	*Aspergillus*	*Aspergillus nidulans*	Aspnid	1	Saprotroph
Fungi	Ascomycota	Eurotiomycetes	Eurotiales	Trichocomaceae	*Aspergillus*	*Aspergillus niger*	Aspni7	1	Saprotroph
Fungi	Ascomycota	Eurotiomycetes	Eurotiales	Trichocomaceae	*Aspergillus*	*Aspergillus niger*	AspniA	1	Saprotroph
Fungi	Ascomycota	Eurotiomycetes	Eurotiales	Trichocomaceae	*Aspergillus*	*Aspergillus niger*	AspniB	1	Saprotroph
Fungi	Ascomycota	Eurotiomycetes	Eurotiales	Trichocomaceae	*Aspergillus*	*Aspergillus niger*	AspniC	1	Saprotroph
Fungi	Ascomycota	Eurotiomycetes	Eurotiales	Trichocomaceae	*Aspergillus*	*Aspergillus novofumigatus*	Aspnov	1	Saprotroph
Fungi	Ascomycota	Eurotiomycetes	Eurotiales	Trichocomaceae	*Aspergillus*	*Aspergillus ochraceoroseus*	Aspoch	1	Saprotroph
Fungi	Ascomycota	Eurotiomycetes	Eurotiales	Trichocomaceae	*Aspergillus*	*Aspergillus oryzae*	Aspor1	1	Saprotroph
Fungi	Ascomycota	Eurotiomycetes	Eurotiales	Trichocomaceae	*Aspergillus*	*Aspergillus steynii*	Aspste	1	Saprotroph
Fungi	Ascomycota	Eurotiomycetes	Eurotiales	Trichocomaceae	*Aspergillus*	*Aspergillus sydowii*	Aspsy1	1	Saprotroph
Fungi	Ascomycota	Eurotiomycetes	Eurotiales	Trichocomaceae	*Aspergillus*	*Aspergillus terreus*	Aspte1	1	Saprotroph
Fungi	Ascomycota	Eurotiomycetes	Eurotiales	Trichocomaceae	*Aspergillus*	*Aspergillus tubingensis*	Asptu1	1	Saprotroph
Fungi	Ascomycota	Eurotiomycetes	Eurotiales	Trichocomaceae	*Aspergillus*	*Aspergillus versicolor*	Aspve1	1	Saprotroph
Fungi	Ascomycota	Eurotiomycetes	Eurotiales	Trichocomaceae	*Aspergillus*	*Aspergillus wentii*	Aspwe1	1	Saprotroph
Fungi	Ascomycota	Eurotiomycetes	Eurotiales	Trichocomaceae	*Aspergillus*	*Aspergillus zonatus*	Aspzo1	1	Saprotroph

Continuation **Table S4**

Kingdom	Phylum	Class	Order	Family	Genus	Species	JGI nomenclature	Number of copies	Ecology
Fungi	Basidiomycota	Atractiellomycetes	Atractiellales			*Atractiellales* sp.	Atrsp2	1	
Fungi	Ascomycota	Dothideomycetes	Microthyriales	Aulographaceae	*Aulographum*	*Aulographum hederae*	Aulhe2	1	Saprotroph
Fungi	Ascomycota	Dothideomycetes	Dothideales	Dothioraceae	*Aureobasidium*	*Aureobasidium pullulans*	AurpuA	1	Saprotroph
Fungi	Ascomycota	Dothideomycetes	Dothideales	Dothioraceae	*Aureobasidium*	*Aureobasidium pullulans*	AurpuB	1	Saprotroph
Fungi	Ascomycota	Dothideomycetes	Dothideales	Dothioraceae	*Aureobasidium*	*Aureobasidium pullulans*	AurpuC	1	Saprotroph
Fungi	Ascomycota	Dothideomycetes	Dothideales	Dothioraceae	*Aureobasidium*	*Aureobasidium pullulans*	AurpuD	1	Saprotroph
Fungi	Basidiomycota	Agaricomycetes	Auriculariales	Auriculariaceae	*Auricularia*	*Auricularia subglabra*	Aurde3	1	Saprotroph
Fungi	Basidiomycota	Agaricomycetes	Russulales	Auriscalpiaceae	*Auriscalpium*	*Auriscalpium vulgare*	Aurvu1	1	Saprotroph
Fungi	Ascomycota	Saccharomycetes	Saccharomycetales	Debaryomycetaceae	*Babjeviella*	*Babjeviella inositovora*	Babin1	1	Saprotroph
Fungi	Zygomycota	Incertae sedis	Mucorales	Backusellaceae	*Backusella*	*Backusella circina*	Bacci1	3	
Fungi	Chytridiomycota	Chytridiomycetes	Rhizophydiales	Incertae sedis	*Batrachochytrium*	*Batrachochytrium dendrobatidis*	Batde5	1	
Fungi	Ascomycota	Dothideomycetes	Capnodiales	Incertae sedis	*Baudoinia*	*Baudoinia compniacensis*	Bauco1	1	
Fungi	Ascomycota	Sordariomycetes	Hypocreales	Cordycipitaceae	*Beauveria*	*Beauveria bassiana*	Beaba1	1	Animal parasite
Fungi	Ascomycota	Dothideomycetes	Pleosporales	Montagnulaceae	*Bimuria*	*Bimuria novae-zelandiae*	Bimnz1	1	Saprotroph
Fungi	Basidiomycota	Agaricomycetes	Polyporales	Meruliaceae	*Bjerkandera*	*Bjerkandera adusta*	Bjead1	1	Saprotroph
Fungi	Ascomycota	Saccharomycetes	Saccharomycetales	Trichomonascaceae	*Blastobotrys*	*Blastobotrys (Arxula)*	Arxad1	1	Saprotroph
Fungi	Ascomycota	Leotiomycetes	Erysiphales	Erysiphaceae	*Blumeria*	*Blumeria graminis*	Blugr1	1	Pathogen
Fungi	Basidiomycota	Agaricomycetes	Boletales	Boletaceae	*Boletus*	*Boletus edulis*	Boled1	1	Ectomycorrhizal
Fungi	Basidiomycota	Agaricomycetes	Cantharellales	Botryobasidiaceae	*Botryobasidium*	*Botryobasidium botryosum*	Botbo1	1	Saprotroph
Fungi	Ascomycota	Dothideomycetes	Botryosphaeriales	Botryosphaeriaceae	*Botryosphaeria*	*Botryosphaeria dothidea*	Botdo1	1	Pathogen
Fungi	Ascomycota	Leotiomycetes	Helotiales	Sclerotiniaceae	*Botrytis*	*Botrytis cinerea*	Botci1	1	Pathogen
Fungi	Ascomycota	Dothideomycetes	Pleosporales	Dacampiaceae	*Byssothecium*	*Byssothecium circinans*	Bysci1	2	Saprotroph
Fungi	Ascomycota	Leotiomycetes	Helotiales	Helotiaceae	*Cadophora*	*Cadophora* sp.	Cadsp1	1	Saprotroph
Fungi	Ascomycota	Eurotiomycetes	Coryneliales	Coryneliaceae	*Caliciopsis*	*Caliciopsis orientalis*	Calor1	1	Pathogen
Fungi	Basidiomycota	Dacrymycetes	Dacrymycetales	Dacrymycetaceae	*Calocera*	*Calocera cornea*	Calco1	1	Saprotroph
Fungi	Basidiomycota	Dacrymycetes	Dacrymycetales	Dacrymycetaceae	*Calocera*	*Calocera viscosa*	Calvi1	1	Saprotroph
Fungi	Ascomycota	Sordariomycetes	Calosphaeriales	Calosphaeriaceae	*Calosphaeria*	*Calosphaeria pulchella*	Calpu1	2	Saprotroph
Fungi	Ascomycota	Saccharomycetes	Saccharomycetales	Incertae sedis	*Candida*	*Candida arabinofermentans*	Canar1	1	
Fungi	Ascomycota	Saccharomycetes	Saccharomycetales	Incertae sedis	*Candida*	*Candida caseinolytica*	Canca1	1	
Fungi	Ascomycota	Saccharomycetes	Saccharomycetales	Incertae sedis	*Candida*	*Candida tanzawaensis*	Canta1	1	
Fungi	Ascomycota	Saccharomycetes	Saccharomycetales	Incertae sedis	*Candida*	*Candida tenuis*	Cante1	1	
Fungi	Chytridiomycota	Blastocladiomycetes	Blastocladiales	Catenariaceae	*Catenaria*	*Catenaria anguillulae*	Catan1	1	Saprotroph
Fungi	Ascomycota	Dothideomycetes	Hysteriales	Gloniaceae	*Cenococcum*	*Cenococcum geophilum*	Cenge3	1	Ectomycorrhizal
Fungi	Ascomycota	Saccharomycetes	Saccharomycetales	Cephaloascaceae	*Cephaloascus*	*Cephaloascus albidus*	Cepal1	1	Saprotroph
Fungi	Ascomycota	Saccharomycetes	Saccharomycetales	Cephaloascaceae	*Cephaloascus*	*Cephaloascus fragrans*	Cepfr1	1	Saprotroph
Fungi	Ascomycota	Dothideomycetes	Capnodiales	Mycosphaerellaceae	*Cercospora*	*Cercospora zeae-maydis*	Cerzm1	1	Pathogen
Fungi	Basidiomycota	Agaricomycetes	Polyporales	Phanerochaetaceae	*Ceriporiopsis*	*Ceriporiopsis (Gelatoporia)*	Cersu1	1	Saprotroph
Fungi	Basidiomycota	Agaricomycetes	Polyporales	Polyporaceae	*Cerrena*	*Cerrena unicolor*	Cerun2	1	Saprotroph
Fungi	Ascomycota	Sordariomycetes	Sordariales	Chaetomiaceae	*Chaetomium*	*Chaetomium globosum*	Chagl_	1	Saprotroph
Fungi	Ascomycota	Sordariomycetes	Chaetosphaeriales	Chaetosphaeriaceae	*Chaetosphaeria*	*Chaetosphaeria innumera*	Chain1	1	Saprotroph
Fungi	Ascomycota	Incertae sedis	Incertae sedis	Incertae sedis	*Chalara*	*Chalara longipes*	Chalo1	1	Saprotroph
Fungi	Basidiomycota	Agaricostilbomycetes	Agaricostilbales	Chionosphaeraceae	*Chionosphaera*	*Chionosphaera apobasidialis*	Chiap1	1	Mycoparasite
Fungi	Ascomycota	Pezizomycetes	Pezizales	Tuberaceae	*Choiromyces*	*Choiromyces venosus*	Chove1	1	Ectomycorrhizal
Fungi	Ascomycota	Lecanoromycetes	Lecanorales	Cladoniaceae	*Cladonia*	*Cladonia grayi*	Clagr3	1	Lichenized
Fungi	Ascomycota	Dothideomycetes	Capnodiales	Davidiellaceae	*Cladosporium*	*Cladosporium fulvum*	Clafu1	1	Saprotroph
Fungi	Ascomycota	Dothideomycetes	Pleosporales	Pleosporaceae	*Clathrospora*	*Clathrospora elynae*	Clael1	2	Pathogen
Fungi	Basidiomycota	Agaricomycetes	Russulales	Auriscalpiaceae	*Clavicorona*	*Clavicorona pyxidata*	Clapy1	1	Saprotroph
Fungi	Ascomycota	Saccharomycetes	Saccharomycetales	Metschnikowiaceae	*Clavispora*	*Clavispora lusitaniae*	Clalu1	1	Saprotroph
Fungi	Basidiomycota	Agaricomycetes	Cantharellales	Clavulinaceae	*Clavulina*	*Clavulina* sp.	ClaPMI	1	Ectomycorrhizal
Fungi	Ascomycota	Eurotiomycetes	Onygenales	Onygenaceae	*Coccidioides*	*Coccidioides immitis*	Cocim1	1	Saprotroph
Fungi	Ascomycota	Dothideomycetes	Pleosporales	Pleosporaceae	*Cochliobolus*	*Cochliobolus carbonum*	Cocca1	2	Pathogen
Fungi	Ascomycota	Dothideomycetes	Pleosporales	Pleosporaceae	*Cochliobolus*	*Cochliobolus heterostrophus*	CocheA	2	Pathogen
Fungi	Ascomycota	Dothideomycetes	Pleosporales	Pleosporaceae	*Cochliobolus*	*Cochliobolus heterostrophus*	CocheB	2	Pathogen
Fungi	Ascomycota	Dothideomycetes	Pleosporales	Pleosporaceae	*Cochliobolus*	*Cochliobolus lunatus*	Coclu2	2	Pathogen
Fungi	Ascomycota	Dothideomycetes	Pleosporales	Pleosporaceae	*Cochliobolus*	*Cochliobolus miyabeanus*	Cocmi1	2	Pathogen
Fungi	Ascomycota	Dothideomycetes	Pleosporales	Pleosporaceae	*Cochliobolus*	*Cochliobolus sativus*	Cocsa1	2	Pathogen
Fungi	Ascomycota	Dothideomycetes	Pleosporales	Pleosporaceae	*Cochliobolus*	*Cochliobolus victoriae*	Cocvi1	2	Pathogen
Fungi	Zygomycota	Incertae sedis	Kickxellales	Kickxellaceae	*Coemansia*	*Coemansia reversa*	Coere1	1	Saprotroph
Fungi	Ascomycota	Sordariomycetes	Hypocreales	Glomerellaceae	*Colletotrichum*	*Colletotrichum eremochloae*	Coler1	1	Pathogen
Fungi	Ascomycota	Sordariomycetes	Hypocreales	Glomerellaceae	*Colletotrichum*	*Colletotrichum falcatum*	Colfa1	1	Pathogen
Fungi	Ascomycota	Sordariomycetes	Hypocreales	Glomerellaceae	*Colletotrichum*	*Colletotrichum graminicola*	Colgr1	1	Pathogen

Continuation **Table S4**

Kingdom	Phylum	Class	Order	Family	Genus	Species	JGI nomenclature	Number of copies	Ecology
Fungi	Ascomycota	Sordariomycetes	Hypocreales	Glomerellaceae	*Colletotrichum*	*Colletotrichum higginsianum*	Colhi1	2	Pathogen
Fungi	Ascomycota	Sordariomycetes	Hypocreales	Glomerellaceae	*Colletotrichum*	*Colletotrichum somersetensis*	Colso1	1	Pathogen
Fungi	Ascomycota	Sordariomycetes	Hypocreales	Glomerellaceae	*Colletotrichum*	*Colletotrichum sublineola*	Colsu1	1	Pathogen
Fungi	Ascomycota	Sordariomycetes	Hypocreales	Glomerellaceae	*Colletotrichum*	*Colletotrichum zoysiae*	Colzo1	1	Pathogen
Fungi	Zygomycota	Incertae sedis	Entomophthorales	Ancylistaceae	*Conidiobolus*	*Conidiobolus coronatus*	Conco1	1	Animal parasite
Fungi	Zygomycota	Incertae sedis	Entomophthorales	Ancylistaceae	*Conidiobolus*	*Conidiobolus thromboides*	Conth1	1	Animal parasite
Fungi	Ascomycota	Sordariomycetes	Coniochaetales	Coniochaetaceae	*Coniochaeta*	*Coniochaeta ligniaria*	Conli1	1	Saprotroph
Fungi	Ascomycota	Sordariomycetes	Coniochaetales	Coniochaetaceae	*Coniochaeta*	*Coniochaeta ligniaria*	Conlig	1	Saprotroph
Fungi	Ascomycota	Sordariomycetes	Coniochaetales	Coniochaetaceae	*Coniochaeta*	*Coniochaeta* sp.	ConPMI	1	Saprotroph
Fungi	Basidiomycota	Agaricomycetes	Boletales	Coniophoraceae	*Coniophora*	*Coniophora puteana*	Conpu1	1	Saprotroph
Fungi	Basidiomycota	Agaricomycetes	Agaricales	Psathyrellaceae	*Coprinopsis*	*Coprinopsis cinerea*	Copci1	1	Saprotroph
Fungi	Basidiomycota	Agaricomycetes	Agaricales	Psathyrellaceae	*Coprinopsis*	*Coprinopsis cinerea*	Copci_	1	Saprotroph
Fungi	Ascomycota	Sordariomycetes	Hypocreales	Cordycipitaceae	*Cordyceps*	*Cordyceps militaris*	Cormi1	1	Animal parasite
Fungi	Basidiomycota	Agaricomycetes	Agaricales	Cortinariaceae	*Cortinarius*	*Cortinarius glaucopus*	Corgl3	1	Ectomycorrhizal
Fungi	Ascomycota	Dothideomycetes	Pleosporales	Corynesporascaceae	*Corynespora*	*Corynespora cassiicola*	Corca1	2	Saprotroph
Fungi	Basidiomycota	Pucciniomycetes	Pucciniales	Cronartiaceae	*Cronartium*	*Cronartium quercuum*	Croqu1	1	Pathogen
Fungi	Ascomycota	Sordariomycetes	Diaporthales	Cryphonectriaceae	*Cryphonectria*	*Cryphonectria parasitica*	Crypa2	1	Pathogen
Fungi	Basidiomycota	Tremellomycetes	Filobasidiales	Filobasidiaceae	*Cryptococcus*	*Cryptococcus neoformans*	CryneA	0	Saprotroph
Fungi	Basidiomycota	Tremellomycetes	Filobasidiales	Filobasidiaceae	*Cryptococcus*	*Cryptococcus neoformans*	CryneB	0	Saprotroph
Fungi	Basidiomycota	Tremellomycetes	Filobasidiales	Filobasidiaceae	*Cryptococcus*	*Cryptococcus vishniacii*	Cryvi1	1	Saprotroph
Fungi	Ascomycota	Dothideomycetes	Pleosporales	Cucurbitariaceae	*Cucurbitaria*	*Cucurbitaria berberidis*	Cucbe1	2	Saprotroph
Fungi	Ascomycota	Saccharomycetes	Saccharomycetales	Incertae sedis	*Cyberlindnera*	*Cyberlindnera jadinii*	Cybja1	1	
Fungi	Basidiomycota	Agaricomycetes	Agaricales	Physalacriaceae	*Cylindrobasidium*	*Cylindrobasidium torrendii*	Cylto1	1	
Fungi	Basidiomycota	Dacrymycetes	Dacrymycetales	Dacrymycetaceae	*Dacryopinax*	*Dacryopinax* sp.	Dacsp1	1	Saprotroph
Fungi	Basidiomycota	Agaricomycetes	Polyporales	Fomitopsidaceae	*Daedalea*	*Daedalea quercina*	Daequ1	1	Saprotroph
Fungi	Ascomycota	Sordariomycetes	Xylariales	Xylariaceae	*Daldinia*	*Daldinia eschscholzii*	DalEC1	1	Saprotroph
Fungi	Ascomycota	Saccharomycetes	Saccharomycetales	Incertae sedis	*Debaryomyces*	*Debaryomyces hansenii*	Debha1	1	
Fungi	Ascomycota	Saccharomycetes	Saccharomycetales	Pichiaceae	*Dekkera*	*Dekkera bruxellensis*	Dekbr2	1	Saprotroph
Fungi	Ascomycota	Dothideomycetes	Pleosporales	Delitschiaceae	*Delitschia*	*Delitschia confertaspora*	Delco1	2	Saprotroph
Fungi	Basidiomycota	Agaricomycetes	Corticiales	Corticiaceae	*Dendrothele*	*Dendrothele bispora*	Denbi1	1	Saprotroph
Fungi	Basidiomycota	Agaricomycetes	Polyporales	Polyporaceae	*Dichomitus*	*Dichomitus squalens*	Dicsq1	1	Saprotroph
Fungi	Ascomycota	Dothideomycetes	Pleosporales	Incertae sedis	*Didymella*	*Didymella exigua*	Didex1	2	Saprotroph
Fungi	Basidiomycota	Tremellomycetes	Tremellales	Incertae sedis	*Dioszegia*	*Dioszegia cryoxerica*	Diocr1	0	Saprotroph
Fungi	Ascomycota	Dothideomycetes	Capnodiales	Mycosphaerellaceae	*Dissoconium*	*Dissoconium aciculare*	Disac1	1	Saprotroph
Fungi	Ascomycota	Dothideomycetes	Pleosporales	Dothidotthiaceae	*Dothidotthia*	*Dothidotthia symphoricarpi*	Dotsy1	2	Pathogen
Fungi	Ascomycota	Dothideomycetes	Capnodiales	Mycosphaerellaceae	*Dothistroma*	*Dothistroma septosporum*	Dotse1	1	Pathogen
Fungi	Microsporidia	Microsporea	Microsporida	Encephalitozoonidae	*Encephalitozoon*	*Encephalitozoon cuniculi*	Enccu1	0	Biotroph
Fungi	Microsporidia	Microsporea	Microsporida	Encephalitozoonidae	*Encephalitozoon*	*Encephalitozoon hellem*	Enche1	0	Biotroph
Fungi	Microsporidia	Microsporea	Microsporida	Encephalitozoonidae	*Encephalitozoon*	*Encephalitozoon intestinalis*	Encin1	0	Biotroph
Fungi	Microsporidia	Microsporea	Microsporida	Encephalitozoonidae	*Encephalitozoon*	*Encephalitozoon romaleae*	Encro1	0	Biotroph
Fungi	Microsporidia	Microsporea	Microsporida	Enterocytozoonidae	*Enterocytozoon*	*Enterocytozoon bieneusi*	Entbi1	0	Biotroph
Fungi	Ascomycota	Dothideomycetes	Incertae sedis	Eremomycetaceae	*Eremomyces*	*Eremomyces bilateralis*	Erebi1	1	Saprotroph
Fungi	Ascomycota	Saccharomycetes	Saccharomycetales	Eremotheciaceae	*Eremothecium*	*Eremothecium gossypii*	AshgoB	1	Pathogen
Fungi	Basidiomycota	Cystobasidiomycetes	Erythrobasidiales	Incertae sedis	*Erythrobasidium*	*Erythrobasidium hasegawianum*	Eryha1	1	Saprotroph
Fungi	Ascomycota	Eurotiomycetes	Eurotiales	Trichocomaceae	*Eurotium*	*Eurotium rubrum*	Eurhe1	1	Saprotroph
Fungi	Ascomycota	Sordariomycetes	Xylariales	Diatrypaceae	*Eutypa*	*Eutypa lata*	Eutla1	2	Pathogen
Fungi	Basidiomycota	Agaricomycetes	Auriculariales	Exidiaceae	*Exidia*	*Exidia glandulosa*	Exigl1	1	Saprotroph
Fungi	Basidiomycota	Exobasidiomycetes	Exobasidiales	Exobasidiaceae	*Exobasidium*	*Exobasidium vaccinii*	Exova1	1	Pathogen
Fungi	Basidiomycota	Agaricomycetes	Polyporales	Fomitopsidaceae	*Fibroporia*	*Fibroporia radiculosa*	Fibra1	1	Saprotroph
Fungi	Basidiomycota	Agaricomycetes	Atheliales	Atheliaceae	*Fibulorhizoctonia*	*Fibulorhizoctonia sp.*	Fibsp1	1	Saprotroph
Fungi	Basidiomycota	Agaricomycetes	Agaricales	Fistulinaceae	*Fistulina*	*Fistulina hepatica*	Fishe1	1	Pathogen
Fungi	Basidiomycota	Agaricomycetes	Hymenochaetales	Hymenochaetaceae	*Fomitiporia*	*Fomitiporia mediterranea*	Fomme1	1	Saprotroph
Fungi	Basidiomycota	Agaricomycetes	Polyporales	Fomitopsidaceae	*Fomitopsis*	*Fomitopsis pinicola*	Fompi3	1	Saprotroph
Fungi	Ascomycota	Sordariomycetes	Hypocreales	Nectriaceae	*Fusarium*	*Fusarium fujikuroi*	Fusfu1	1	Pathogen
Fungi	Ascomycota	Sordariomycetes	Hypocreales	Nectriaceae	*Fusarium*	*Fusarium graminearum*	Fusgr1	1	Pathogen
Fungi	Ascomycota	Sordariomycetes	Hypocreales	Nectriaceae	*Fusarium*	*Fusarium oxysporum*	Fusox1	1	Pathogen
Fungi	Ascomycota	Sordariomycetes	Hypocreales	Nectriaceae	*Fusarium*	*Fusarium verticillioides*	Fusve1	1	Pathogen
Fungi	Basidiomycota	Agaricomycetes	Agaricales	Strophariaceae	*Galerina*	*Galerina marginata*	Galma1	1	Saprotroph
Fungi	Basidiomycota	Agaricomycetes	Polyporales	Ganodermataceae	*Ganoderma*	*Ganoderma* sp.	Gansp1	1	Pathogen
Fungi	Ascomycota	Leotiomycetes	Helotiales	Helotiaceae	*Glarea*	*Glarea lozoyensis*	Glalo1	1	Saprotroph
Fungi	Basidiomycota	Agaricomycetes	Gloeophyllales	Gloeophyllaceae	*Gloeophyllum*	*Gloeophyllum trabeum*	Glotr1	1	Saprotroph

Continuation **Table S4**

Kingdom	Phylum	Class	Order	Family	Genus	Species	JGI nomenclature	Number of copies	Ecology
Fungi	Ascomycota	Sordariomycetes	Hypocreales	Glomerellaceae	*Glomerella*	*Glomerella acutata*	Gloac1	1	Pathogen
Fungi	Ascomycota	Sordariomycetes	Hypocreales	Glomerellaceae	*Glomerella*	*Glomerella cingulata*	Gloci1	1	Pathogen
Fungi	Ascomycota	Dothideomycetes	Hysteriales	Gloniaceae	*Glonium*	*Glonium stellatum*	Glost2	1	Saprotroph
Fungi	Chytridiomycota	Monoblepharidomycetes	Monoblepharidales	Gonapodyaceae	*Gonapodya*	*Gonapodya prolifera*	Ganpr1	1	
Fungi	Ascomycota	Sordariomycetes	Ophiostomatales	Ophiostomataceae	*Grosmannia*	*Grosmannia clavigera*	Grocl1	1	Pathogen
Fungi	Basidiomycota	Agaricomycetes	Agaricales	Physalacriaceae	*Guyanagaster*	*Guyanagaster necrorhiza*	Guyne1	1	
Fungi	Ascomycota	Eurotiomycetes	Onygenales	Gymnoascaceae	*Gymnascella*	*Gymnascella aurantiaca*	Gymau1	1	Saprotroph
Fungi	Ascomycota	Eurotiomycetes	Onygenales	Gymnoascaceae	*Gymnascella*	*Gymnascella citrina*	Gymci1	1	Saprotroph
Fungi	Basidiomycota	Agaricomycetes	Agaricales	Strophariaceae	*Gymnopilus*	*Gymnopilus chrysopellus*	Gymch1	1	Saprotroph
Fungi	Basidiomycota	Agaricomycetes	Agaricales	Marasmiaceae	*Gymnopus*	*Gymnopus androsaceus*	Gyman1	1	Saprotroph
Fungi	Basidiomycota	Agaricomycetes	Agaricales	Marasmiaceae	*Gymnopus*	*Gymnopus luxurians*	Gymlu1	1	Saprotroph
Fungi	Basidiomycota	Agaricomycetes	Boletales	Paxillaceae	*Gyrodon*	*Gyrodon lividus*	Gyrli1	1	Ectomycorrhizal
Fungi	Ascomycota	Saccharomycetes	Saccharomycetales	Saccharomycodaceae	*Hanseniaspora*	*Hanseniaspora valbyensis*	Hanva1	1	Saprotroph
Fungi	Ascomycota	Saccharomycetes	Saccharomycetales	Saccharomycetaceae	*Hansenula*	*Hansenula polymorpha*	Hanpo2	1	Saprotroph
Fungi	Basidiomycota	Agaricomycetes	Agaricales	Strophariaceae	*Hebeloma*	*Hebeloma cylindrosporum*	Hebcy2	1	Ectomycorrhizal
Fungi	Zygomycota	Incertae sedis	Mucorales	Cunninghamellaceae	*Hesseltinella*	*Hesseltinella vesiculosa*	Hesve1	1	Saprotroph
Fungi	Basidiomycota	Agaricomycetes	Russulales	Bondarzewiaceae	*Heterobasidion*	*Heterobasidion annosum*	Hetan2	1	Saprotroph
Fungi	Basidiomycota	Microbotryomycetes	Heterogastridiales	Heterogastridiaceae	*Heterogastridium*	*Heterogastridium pycnidioideum*	Hetpy1	1	Mycoparasite
Fungi	Ascomycota	Eurotiomycetes	Onygenales	Ajellomycetaceae	*Histoplasma*	*Histoplasma capsulatum*	Hisca1	1	Saprotroph
Fungi	Basidiomycota	Agaricomycetes	Boletales	Paxillaceae	*Hydnomerulius*	*Hydnomerulius pinastri*	Hydpi2	1	Saprotroph
Fungi	Basidiomycota	Agaricomycetes	Agaricales	Strophariaceae	*Hypholoma*	*Hypholoma sublateritium*	Hypsu1	1	Saprotroph
Fungi	Ascomycota	Saccharomycetes	Saccharomycetales	Pichiaceae	*Hyphopichia*	*Hyphopichia burtonii*	Hypbu1	1	Saprotroph
Fungi	Ascomycota	Sordariomycetes	Xylariales	Xylariaceae	*Hypoxylon*	*Hypoxylon* sp.	HypCI4	1	Saprotroph
Fungi	Ascomycota	Sordariomycetes	Xylariales	Xylariaceae	*Hypoxylon*	*Hypoxylon* sp.	HypCO2	1	Saprotroph
Fungi	Ascomycota	Sordariomycetes	Xylariales	Xylariaceae	*Hypoxylon*	*Hypoxylon* sp.	HypEC3	1	Saprotroph
Fungi	Ascomycota	Dothideomycetes	Hysteriales	Hysteriaceae	*Hysterium*	*Hysterium pulicare*	Hyspu1	2	Saprotroph
Fungi	Ascomycota	Sordariomycetes	Hypocreales	Nectriaceae	*Ilyonectria*	*Ilyonectria sp.*	Ilysp1	1	Pathogen
Fungi	Basidiomycota	Agaricomycetes	Jaapiales	Incertae sedis	*Jaapia*	*Jaapia argillacea*	Jaaar1	1	Saprotroph
Fungi	Basidiomycota	Exobasidiomycetes	Microstromatales	Incertae sedis	*Jaminaea*	*Jaminaea sp.*	Jamsp1	0	
Fungi	Ascomycota	Dothideomycetes	Pleosporales	Melanommataceae	*Karstenula*	*Karstenula rhodostoma*	Karrh1	1	Saprotroph
Fungi	Ascomycota	Saccharomycetes	Saccharomycetales	Saccharomycetaceae	*Kazachstania*	*Kazachstania africana*	Kazaf1	1	Saprotroph
Fungi	Ascomycota	Saccharomycetes	Saccharomycetales	Saccharomycetaceae	*Kluyveromyces*	*Kluyveromyces lactis*	Klula1	1	Saprotroph
Fungi	Basidiomycota	Agaricomycetes	Agaricales	Hydnangiaceae	*Laccaria*	*Laccaria amethystina*	Lacam1	1	Ectomycorrhizal
Fungi	Basidiomycota	Agaricomycetes	Agaricales	Hydnangiaceae	*Laccaria*	*Laccaria bicolor*	Lacbi2	1	Ectomycorrhizal
Fungi	Basidiomycota	Agaricomycetes	Polyporales	Fomitopsidaceae	*Laetiporus*	*Laetiporus sulphureus*	Laesu1	1	Saprotroph
Fungi	Basidiomycota	Incertae sedis	Incertae sedis	Incertae sedis	*Leiotrametes*	*Leiotrametes sp*	Leisp1	1	
Fungi	Basidiomycota	Agaricomycetes	Polyporales	Polyporaceae	*Lentinus*	*Lentinus tigrinus*	Lenti6	1	Saprotroph
Fungi	Basidiomycota	Agaricomycetes	Polyporales	Polyporaceae	*Lentinus*	*Lentinus tigrinus*	Lenti7	1	Saprotroph
Fungi	Basidiomycota	Agaricomycetes	Polyporales	Polyporaceae	*Lentinus*	*Lentinus tigrinus*	Sisbr1	1	Saprotroph
Fungi	Ascomycota	Dothideomycetes	Pleosporales	Incertae sedis	*Lentithecium*	*Lentithecium fluviatile*	Lenfl1	2	
Fungi	Ascomycota	Dothideomycetes	Incertae sedis	Argynnaceae	*Lepidopterella*	*Lepidopterella palustris*	Leppa1	1	Saprotroph
Fungi	Ascomycota	Dothideomycetes	Pleosporales	Leptosphaeriaceae	*Leptosphaeria*	*Leptosphaeria maculans*	Lepmu1	3	Pathogen
Fungi	Basidiomycota	Agaricomycetes	Agaricales	Agaricaceae	*Leucoagaricus*	*Leucoagaricus gongylophorus*	Leugo1	2	Saprotroph
Fungi	Basidiomycota	Agaricomycetes	Boletales	Hygrophoropsidaceae	*Leucogyrophana*	*Leucogyrophana mollusca*	Leumo1	1	Saprotroph
Fungi	Zygomycota	Incertae sedis	Mucorales	Lichtheimiaceae	*Lichtheimia*	*Lichtheimia hyalospora*	Lichy1	1	Saprotroph
Fungi	Ascomycota	Dothideomycetes	Pleosporales	Lindgomycetaceae	*Lindgomyces*	*Lindgomyces ingoldianus*	Linin1	2	
Fungi	Ascomycota	Saccharomycetes	Saccharomycetales	Lipomycetaceae	*Lipomyces*	*Lipomyces starkeyi*	Lipst1	1	Saprotroph
Fungi	Ascomycota	Dothideomycetes	Pleosporales	Lophiostomataceae	*Lophiostoma*	*Lophiostoma macrostomum*	Lopma1	1	Saprotroph
Fungi	Ascomycota	Dothideomycetes	Pleosporales	Mytilinidiaceae	*Lophium*	*Lophium mytilinum*	Lopmy1	1	Saprotroph
Fungi	Ascomycota	Leotiomycetes	Helotiales	Loramycetaceae	*Loramyces*	*Loramyces juncicola*	Lorju1	1	Saprotroph
Fungi	Basidiomycota	Agaricomycetes	Agaricales	Agaricaceae	*Macrolepiota*	*Macrolepiota fuliginosa*	Macfu1	1	Saprotroph
Fungi	Ascomycota	Dothideomycetes	Botryosphaeriales	Botryosphaeriaceae	*Macrophomina*	*Macrophomina phaseolina*	Macph1	1	Pathogen
Fungi	Ascomycota	Dothideomycetes	Pleosporales	Incertae sedis	*Macroventuria*	*Macroventuria anomochaeta*	Macan1	2	
Fungi	Ascomycota	Sordariomycetes	Incertae sedis	Magnaporthaceae	*Magnaporthe*	*Magnaporthe grisea*	Maggr1	2	Pathogen
Fungi	Basidiomycota	Exobasidiomycetes	Malasseziales	Incertae sedis	*Malassezia*	*Malassezia globosa*	Malgl1	1	Saprotroph
Fungi	Basidiomycota	Exobasidiomycetes	Malasseziales	Incertae sedis	*Malassezia*	*Malassezia sympodialis*	Malsy1	1	Saprotroph
Fungi	Basidiomycota	Agaricomycetes	Agaricales	Marasmiaceae	*Marasmius*	*Marasmius fiardii*	Marfi1	1	Saprotroph
Fungi	Ascomycota	Leotiomycetes	Helotiales	Dermateaceae	*Marssonina*	*Marssonina brunnea*	Marbr1	1	Pathogen
Fungi	Zygomycota	Incertae sedis	Kickxellales	Kickxellaceae	*Martensiomyces*	*Martensiomyces pterosporus*	Marpt1	1	Saprotroph
Fungi	Ascomycota	Dothideomycetes	Pleosporales	Massarinaceae	*Massarina*	*Massarina eburnea*	Maseb1	2	Saprotroph
Fungi	Basidiomycota	Exobasidiomycetes	Incertae sedis	Incertae sedis	*Meira*	*Meira miltonrushii*	Meimi1	1	

Continuation **Table S4**

Kingdom	Phylum	Class	Order	Family	Genus	Species	JGI nomenclature	Number of copies	Ecology
Fungi	Basidiomycota	Pucciniomycetes	Pucciniales	Melampsoraceae	*Melampsora*	*Melampsora laricis-populina*	Mellp1	1	Pathogen
Fungi	Ascomycota	Sordariomycetes	Diaporthales	Melanconidaceae	*Melanconium*	*Melanconium* sp.	Melsp1	1	Pathogen
Fungi	Ascomycota	Dothideomycetes	Pleosporales	Melanommataceae	*Melanomma*	*Melanomma pulvis-pyrius*	Melpu1	2	Saprotroph
Fungi	Ascomycota	Leotiomycetes	Incertae sedis	Incertae sedis	*Meliniomyces*	*Meliniomyces bicolor*	Melbi2	1	Saprotroph
Fungi	Ascomycota	Leotiomycetes	Incertae sedis	Incertae sedis	*Meliniomyces*	*Meliniomyces variabilis*	Melva1	1	Saprotroph
Fungi	Ascomycota	Sordariomycetes	Hypocreales	Clavicipitaceae	*Metarhizium*	*Metarhizium acridum*	Metac1	1	Animal parasite
Fungi	Ascomycota	Sordariomycetes	Hypocreales	Clavicipitaceae	*Metarhizium*	*Metarhizium robertsii*	Metan1	1	Animal parasite
Fungi	Ascomycota	Saccharomycetes	Saccharomycetales	Metschnikowiaceae	*Metschnikowia*	*Metschnikowia bicuspidata*	Metbi1	1	Saprotroph
Fungi	Ascomycota	Sordariomycetes	Microascales	Microascaceae	*Microascus*	*Microascus trigonosporus*	Mictr1	2	Saprotroph
Fungi	Ascomycota	Eurotiomycetes	Onygenales	Arthrodermataceae	*Microsporum*	*Microsporum canis*	Micca1	1	Saprotroph
Fungi	Basidiomycota	Microbotryomycetes	Microstromatales			*Microstromatales* sp.	Rhodsp	0	
Fungi	Basidiomycota	Mixiomycetes	Mixiales	Mixiaceae	*Mixia*	*Mixia osmundae*	Mixos1	1	Pathogen
Fungi	Ascomycota	Orbiliomycetes	Orbiliales	Orbiliaceae	*Monacrosporium*	*Monacrosporium haptotylum*	Monha1	1	Saprotroph
Fungi	Ascomycota	Eurotiomycetes	Incertae sedis	Monascaceae	*Monascus*	*Monascus purpureus*	Monpu1	1	Saprotroph
Fungi	Ascomycota	Eurotiomycetes	Incertae sedis	Monascaceae	*Monascus*	*Monascus ruber*	Monru1	1	Saprotroph
Fungi	Basidiomycota	Agaricomycetes	Agaricales	Marasmiaceae	*Moniliophthora*	*Moniliophthora perniciosa*	Monpe1	0	Saprotroph
Fungi	Ascomycota	Pezizomycetes	Pezizales	Morchellaceae	*Morchella*	*Morchella conica*	Morco1	1	Saprotroph
Fungi	Zygomycota	Incertae sedis	Mortierellales	Mortierellaceae	*Mortierella*	*Mortierella elongata*	Morel1	1	Saprotroph
Fungi	Zygomycota	Incertae sedis	Mucorales	Mucoraceae	*Mucor*	*Mucor circinelloides*	Mucci2	1	Saprotroph
Fungi	Ascomycota	Eurotiomycetes	Onygenales	Incertae sedis	*Myceliophthora*	*Myceliophthora thermophila*	Spoth2	1	
Fungi	Ascomycota	Dothideomycetes	Capnodiales	Mycosphaerellaceae	*Mycosphaerella*	*Mycosphaerella fijiensis*	Mycfi2	1	Pathogen
Fungi	Ascomycota	Dothideomycetes	Capnodiales	Mycosphaerellaceae	*Mycosphaerella*	*Mycosphaerella graminicola*	Mycgr3	1	Pathogen
Fungi	Ascomycota	Dothideomycetes	Myriangiales	Myriangiaceae	*Myriangium*	*Myriangium duriaei*	Myrdu1	1	Animal parasite
Fungi	Ascomycota	Sordariomycetes	Hypocreales	Incertae sedis	*Myrothecium*	*Myrothecium inundatum*	Myrin1	1	Saprotroph
Fungi	Ascomycota	Dothideomycetes	Pleosporales	Mytilinidiaceae	*Mytilinidion*	*Mytilinidion resinicola*	Mytre1	1	Saprotroph
Fungi	Ascomycota	Saccharomycetes	Saccharomycetales	Incertae sedis	*Nadsonia*	*Nadsonia fulvescens*	Nadfu1	1	
Fungi	Basidiomycota	Ustilaginomycetes	Ustilaginales	Cintractiellaceae	*Naiadella*	*Naiadella fluitans*	Naifl1	2	Pathogen
Fungi	Ascomycota	Sordariomycetes	Hypocreales	Nectriaceae	*Nectria*	*Nectria haematococca*	Necha2	1	Pathogen
Fungi	Microsporidia				*Nematocida*	*Nematocida parisii*	Nempa1	0	Pathogen
Fungi	Ascomycota	Dothideomycetes	Botryosphaeriales	Botryosphaeriaceae	*Neofusicoccum*	*Neofusicoccum parvum*	Neopa1	1	Pathogen
Fungi	Basidiomycota	Agaricomycetes	Gloeophyllales		*Neolentinus*	*Neolentinus lepideus*	Neole1	1	Saprotroph
Fungi	Ascomycota	Eurotiomycetes	Eurotiales	Trichocomaceae	*Neosartorya*	*Neosartorya fischeri*	Neofi1	1	Saprotroph
Fungi	Ascomycota	Sordariomycetes	Sordariales	Sordariaceae	*Neurospora*	*Neurospora crassa*	Neucr2	1	Saprotroph
Fungi	Ascomycota	Sordariomycetes	Sordariales	Sordariaceae	*Neurospora*	*Neurospora crassa*	Neucr_	2	Saprotroph
Fungi	Ascomycota	Sordariomycetes	Sordariales	Sordariaceae	*Neurospora*	*Neurospora discreta*	Neudi1	1	Saprotroph
Fungi	Ascomycota	Sordariomycetes	Sordariales	Sordariaceae	*Neurospora*	*Neurospora tetrasperma*	NeuteA	1	Saprotroph
Fungi	Ascomycota	Sordariomycetes	Sordariales	Sordariaceae	*Neurospora*	*Neurospora tetrasperma*	NeuteB	1	Saprotroph
Fungi	Ascomycota	Sordariomycetes	Hypocreales	Niessliaceae	*Niesslia*	*Niesslia exilis*	Nieex1	1	Saprotroph
Fungi	Microsporidia	Microsporea	Microsporida	Nosematidae	*Nosema*	*Nosema ceranae*	Nosce1	0	Biotroph
Fungi	Basidiomycota	Agaricomycetes	Polyporales	Incertae sedis	*Obba*	*Obba rivulosa*	Obbri1	1	Saprotroph
Fungi	Ascomycota	Leotiomycetes	Incertae sedis	Myxotrichaceae	*Oidiodendron*	*Oidiodendron maius*	Oidma1	1	Saprotroph
Fungi	Basidiomycota	Agaricomycetes	Agaricales	Marasmiaceae	*Omphalotus*	*Omphalotus olearius*	Ompol1	1	Saprotroph
Fungi	Ascomycota	Dothideomycetes	Pleosporales	Leptosphaeriaceae	*Ophiobolus*	*Ophiobolus disseminans*	Ophdi1	3	Saprotroph
Fungi	Ascomycota	Sordariomycetes	Ophiostomatales	Ophiostomataceae	*Ophiostoma*	*Ophiostoma piceae*	Ophpc1	1	Pathogen
Fungi	Ascomycota	Sordariomycetes	Ophiostomatales	Ophiostomataceae	*Ophiostoma*	*Ophiostoma piliferum*	Ophpi1	1	Pathogen
Fungi	Chytridiomycota	Neocallimastigomycetes	Neocallimastigales	Neocallimastigaceae	*Orpinomyces*	*Orpinomyces* sp	Orpsp1	0	Animal gut
Fungi	Ascomycota	Saccharomycetes	Saccharomycetales	Incertae sedis	*Pachysolen*	*Pachysolen tannophilus*	Pacta1	1	
Fungi	Basidiomycota	Agaricomycetes	Agaricales	Mycenaceae	*Panellus*	*Panellus stipticus*	Panst_	1	Saprotroph
Fungi	Basidiomycota	Agaricomycetes	Polyporales	Polyporaceae	*Panus*	*Panus rudis*	Panru1	1	Saprotroph
Fungi	Ascomycota	Eurotiomycetes	Onygenales	Ajellomycetaceae	*Paracoccidioides*	*Paracoccidioides brasiliensis*	Parbr1	1	Saprotroph
Fungi	Ascomycota	Dothideomycetes	Pleosporales	Montagnulaceae	*Paraconiothyrium*	*Paraconiothyrium sporulosum*	Parsp1	1	Saprotroph
Fungi	Ascomycota	Dothideomycetes	Patellariales	Patellariaceae	*Patellaria*	*Patellaria atrata*	Patat1	1	Saprotroph
Fungi	Basidiomycota	Agaricomycetes	Boletales	Paxillaceae	*Paxillus*	*Paxillus involutus*	Paxin1	1	Ectomycorrhizal
Fungi	Basidiomycota	Agaricomycetes	Boletales	Paxillaceae	*Paxillus*	*Paxillus rubicundulus*	Paxru1	1	Ectomycorrhizal
Fungi	Ascomycota	Eurotiomycetes	Eurotiales	Trichocomaceae	*Penicillium*	*Penicillium bilaiae*	Penbi1	1	Saprotroph
Fungi	Ascomycota	Eurotiomycetes	Eurotiales	Trichocomaceae	*Penicillium*	*Penicillium brevicompactum*	Penbr2	1	Saprotroph
Fungi	Ascomycota	Eurotiomycetes	Eurotiales	Trichocomaceae	*Penicillium*	*Penicillium brevicompactum*	PenbrA	1	Saprotroph
Fungi	Ascomycota	Eurotiomycetes	Eurotiales	Trichocomaceae	*Penicillium*	*Penicillium canescens*	Penca1	1	Saprotroph
Fungi	Ascomycota	Eurotiomycetes	Eurotiales	Trichocomaceae	*Penicillium*	*Penicillium chrysogenum*	Pench1	1	Saprotroph
Fungi	Ascomycota	Eurotiomycetes	Eurotiales	Trichocomaceae	*Penicillium*	*Penicillium chrysogenum*	PenchW	1	Saprotroph
Fungi	Ascomycota	Eurotiomycetes	Eurotiales	Trichocomaceae	*Penicillium*	*Penicillium digitatum*	Pendi1	1	Saprotroph

Continuation **Table S4**

Kingdom	Phylum	Class	Order	Family	Genus	Species	JGI nomenclature	Number of copies	Ecology
Fungi	Ascomycota	Eurotiomycetes	Eurotiales	Trichocomaceae	*Penicillium*	*Penicillium expansum*	Penex1	1	Saprotroph
Fungi	Ascomycota	Eurotiomycetes	Eurotiales	Trichocomaceae	*Penicillium*	*Penicillium fellutanum*	Penfe1	1	Saprotroph
Fungi	Ascomycota	Eurotiomycetes	Eurotiales	Trichocomaceae	*Penicillium*	*Penicillium glabrum*	Pengl1	1	Saprotroph
Fungi	Ascomycota	Eurotiomycetes	Eurotiales	Trichocomaceae	*Penicillium*	*Penicillium janthinellum*	Penja1	1	Saprotroph
Fungi	Ascomycota	Eurotiomycetes	Eurotiales	Trichocomaceae	*Penicillium*	*Penicillium lanosocoeruleum*	Penla1	1	Saprotroph
Fungi	Ascomycota	Eurotiomycetes	Eurotiales	Trichocomaceae	*Penicillium*	*Penicillium oxalicum*	Penox1	1	Saprotroph
Fungi	Ascomycota	Eurotiomycetes	Eurotiales	Trichocomaceae	*Penicillium*	*Penicillium raistrickii*	Penra1	1	Saprotroph
Fungi	Ascomycota	Dothideomycetes	Pleosporales	Incertae sedis	*Periconia*	*Periconia macrospinosa*	Perma1	2	
Fungi	Ascomycota	Sordariomycetes	Diaporthales	Togniniaceae	*Phaeoacremonium*	*Phaeoacremonium aleophilum*	Phaal1	2	Pathogen
Fungi	Basidiomycota	Agaricomycetes	Polyporales	Phanerochaetaceae	*Phanerochaete*	*Phanerochaete carnosa*	Phaca1	1	Saprotroph
Fungi	Basidiomycota	Agaricomycetes	Polyporales	Phanerochaetaceae	*Phanerochaete*	*Phanerochaete chrysosporium*	Phchr2	1	Saprotroph
Fungi	Basidiomycota	Agaricomycetes	Polyporales	Meruliaceae	*Phlebia*	*Phlebia brevispora*	Phlbr1	1	Saprotroph
Fungi	Basidiomycota	Agaricomycetes	Polyporales	Phanerochaetaceae	*Phlebiopsis*	*Phlebiopsis gigantea*	Phlgi1	1	Saprotroph
Fungi	Ascomycota	Dothideomycetes	Pleosporales	Didymellaceae	*Phoma*	*Phoma tracheiphila*	Photr1	2	Pathogen
Fungi	Zygomycota	Incertae sedis	Mucorales	Phycomycetaceae	*Phycomyces*	*Phycomyces blakesleeanus*	Phybl2	1	Saprotroph
Fungi	Ascomycota	Dothideomycetes	Botryosphaeriales	Phyllostictaceae	*Phyllosticta*	*Phyllosticta citriasiana*	Phycit	1	Pathogen
Fungi	Ascomycota	Saccharomycetes	Saccharomycetales	Pichiaceae	*Pichia*	*Pichia membranifaciens*	Picme2	1	Saprotroph
Fungi	Ascomycota	Saccharomycetes	Saccharomycetales	Pichiaceae	*Pichia*	*Pichia pastoris*	Picpa1	1	Saprotroph
Fungi	Ascomycota	Saccharomycetes	Saccharomycetales	Pichiaceae	*Pichia*	*Pichia stipitis*	Picst3	1	Saprotroph
Fungi	Ascomycota	Dothideomycetes	Capnodiales	Piedraiaceae	*Piedraia*	*Piedraia hortae*	Pieho1	1	Animal parasite
Fungi	Basidiomycota	Agaricomycetes	Atheliales	Atheliaceae	*Piloderma*	*Piloderma croceum*	Pilcr1	1	Ectomycorrhizal
Fungi	Basidiomycota	Agaricomycetes	Sebacinales	Sebacinales Group B	*Piriformospora*	*Piriformospora indica*	Pirin1	1	Saprotroph
Fungi	Chytridiomycota	Neocallimastigomycetes	Neocallimastigales	Neocallimastigaceae	*Piromyces*	*Piromyces sp.*	PirE2_	0	Animal gut
Fungi	Basidiomycota	Agaricomycetes	Boletales	Sclerodermataceae	*Pisolithus*	*Pisolithus microcarpus*	Pismi1	1	Ectomycorrhizal
Fungi	Basidiomycota	Agaricomycetes	Boletales	Sclerodermataceae	*Pisolithus*	*Pisolithus tinctorius*	Pisti1	1	Ectomycorrhizal
Fungi	Ascomycota	Dothideomycetes	Pleosporales	Pleomassariaceae	*Pleomassaria*	*Pleomassaria siparia*	Plesi1	2	Saprotroph
Fungi	Basidiomycota	Agaricomycetes	Agaricales	Pleurotaceae	*Pleurotus*	*Pleurotus ostreatus*	PleosA	1	Saprotroph
Fungi	Basidiomycota	Agaricomycetes	Agaricales	Pleurotaceae	*Pleurotus*	*Pleurotus ostreatus*	PleosB	1	Saprotroph
Fungi	Basidiomycota	Agaricomycetes	Amylocorticiales	Incertae sedis	*Plicaturopsis*	*Plicaturopsis crispa*	Plicr1	1	Saprotroph
Fungi	Ascomycota	Pneumocystidomycetes	Pneumocystidales	Pneumocystidaceae	*Pneumocystis*	*Pneumocystis jirovecii*	Pneji1	1	Animal parasite
Fungi	Ascomycota	Sordariomycetes	Sordariales	Lasiosphaeriaceae	*Podospora*	*Podospora anserina*	Podan2	1	Saprotroph
Fungi	Ascomycota	Dothideomycetes	Capnodiales	Capnodiaceae	*Polychaeton*	*Polychaeton citri*	Polci1	1	Saprotroph
Fungi	Ascomycota	Dothideomycetes	Pleosporales	Tetraplosphaeriaceae	*Polyplosphaeria*	*Polyplosphaeria fusca*	Polfu1	3	Saprotroph
Fungi	Basidiomycota	Agaricomycetes	Polyporales	Polyporaceae	*Polyporus*	*Polyporus arcularius*	Polar1	1	Saprotroph
Fungi	Basidiomycota	Agaricomycetes	Polyporales	Fomitopsidaceae	*Postia*	*Postia placenta*	Pospl1	1	Saprotroph
Fungi	Basidiomycota	Agaricomycetes	Polyporales	Fomitopsidaceae	*Postia*	*Postia placenta*	PosplR	2	Saprotroph
Fungi	Ascomycota	Taphrinomycetes	Taphrinales	Protomycetaceae	*Protomyces*	*Protomyces inouyei*	Proin1	1	Pathogen
Fungi	Ascomycota	Incertae sedis	Triblidiales	Triblidiaceae	*Pseudographis*	*Pseudographis elatina*	Pseel1	1	Saprotroph
Fungi	Ascomycota	Dothideomycetes	Capnodiales	Incertae sedis	*Pseudovirgaria*	*Pseudovirgaria hyperparasitica*	Psehy1	1	
Fungi	Basidiomycota	Ustilaginomycetes	Ustilaginales	Ustilaginaceae	*Pseudozyma*	*Pseudozyma antarctica*	Psean1	1	Pathogen
Fungi	Basidiomycota	Ustilaginomycetes	Ustilaginales	Ustilaginaceae	*Pseudozyma*	*Pseudozyma hubeiensis*	Psehu1	1	Pathogen
Fungi	Basidiomycota	Pucciniomycetes	Pucciniales	Pucciniaceae	*Puccinia*	*Puccinia graminis*	Pucgr1	1	Pathogen
Fungi	Basidiomycota	Pucciniomycetes	Pucciniales	Pucciniaceae	*Puccinia*	*Puccinia striiformis*	Pucst1	1	Pathogen
Fungi	Basidiomycota	Pucciniomycetes	Pucciniales	Pucciniaceae	*Puccinia*	*Puccinia triticina*	Puctr1	1	Pathogen
Fungi	Basidiomycota	Agaricomycetes	Corticiales	Corticiaceae	*Punctularia*	*Punctularia strigosozonata*	Punst1	1	Saprotroph
Fungi	Basidiomycota	Agaricomycetes	Polyporales	Polyporaceae	*Pycnoporus*	*Pycnoporus cinnabarinus*	Pycci1	1	Saprotroph
Fungi	Basidiomycota	Agaricomycetes	Polyporales	Polyporaceae	*Pycnoporus*	*Pycnoporus coccineus*	PyccoA	1	Saprotroph
Fungi	Basidiomycota	Agaricomycetes	Polyporales	Polyporaceae	*Pycnoporus*	*Pycnoporus coccineus*	PyccoB	1	Saprotroph
Fungi	Basidiomycota	Agaricomycetes	Polyporales	Polyporaceae	*Pycnoporus*	*Pycnoporus sanguineus*	Pycsa1	1	Saprotroph
Fungi	Ascomycota	Dothideomycetes	Pleosporales	Leptosphaeriaceae	*Pyrenochaeta*	*Pyrenochaeta* sp.	Pyrsp1	2	Saprotroph
Fungi	Ascomycota	Dothideomycetes	Pleosporales	Pleosporaceae	*Pyrenophora*	*Pyrenophora teres*	Pyrtt1	2	Pathogen
Fungi	Ascomycota	Dothideomycetes	Pleosporales	Pleosporaceae	*Pyrenophora*	*Pyrenophora tritici-repentis*	Pyrtr1	2	Pathogen
Fungi	Ascomycota	Pezizomycetes	Pezizales	Pyronemataceae	*Pyronema*	*Pyronema confluens*	Pyrco1	1	Saprotroph
Fungi	Basidiomycota	Agaricomycetes	Gomphales	Gomphaceae	*Ramaria*	*Ramaria rubella*	Ramac1	1	Ectomycorrhizal
Fungi	Zygomycota	Incertae sedis	Kickxellales	Kickxellaceae	*Ramicandelaber*	*Ramicandelaber brevisporus*	Rambr1	1	Saprotroph
Fungi	Basidiomycota	Agaricomycetes	Cantharellales	Ceratobasidiaceae	*Rhizoctonia*	*Rhizoctonia solani*	Rhiso1	1	
Fungi	Glomeromycota	Glomeromycetes	Glomerales	Glomeraceae	*Rhizophagus*	*Rhizophagus irregularis*	Gloin1	2	Endomycorhizal
Fungi	Basidiomycota	Agaricomycetes	Boletales	Rhizopogonaceae	*Rhizopogon*	*Rhizopogon salebrosus*	Rhisa1	1	Ectomycorrhizal
Fungi	Basidiomycota	Agaricomycetes	Boletales	Rhizopogonaceae	*Rhizopogon*	*Rhizopogon vinicolor*	Rhivi1	1	Ectomycorrhizal
Fungi	Zygomycota	Incertae sedis	Mucorales	Rhizopodaceae	*Rhizopus*	*Rhizopus microsporus*	Rhich1	1	Saprotroph
Fungi	Zygomycota	Incertae sedis	Mucorales	Rhizopodaceae	*Rhizopus*	*Rhizopus microsporus*	Rhimi1	1	Saprotroph

Continuation **Table S4**

Kingdom	Phylum	Class	Order	Family	Genus	Species	JGI nomenclature	Number of copies	Ecology
Fungi	Zygomycota	Incertae sedis	Mucorales	Rhizopodaceae	*Rhizopus*	*Rhizopus oryzae*	Rhior3	2	Saprotroph
Fungi	Ascomycota	Leotiomycetes	Helotiales	Helotiaceae	*Rhizoscyphus*	*Rhizoscyphus ericae*	Rhier1	1	Saprotroph
Fungi	Basidiomycota	Microbotryomycetes	Sporidiobolales	Incertae sedis	*Rhodosporidium*	*Rhodosporidium toruloides*	Rhoto1	1	Mycoparasite
Fungi	Basidiomycota	Microbotryomycetes	Sporidiobolales	Incertae sedis	*Rhodotorula*	*Rhodotorula graminis*	Rhoba1	1	Saprotroph
Fungi	Basidiomycota	Microbotryomycetes	Sporidiobolales	Incertae sedis	*Rhodotorula*	*Rhodotorula minuta*	Rhomi1	1	Saprotroph
Fungi	Basidiomycota	Microbotryomycetes	Sporidiobolales	Incertae sedis	*Rhodotorula*	*Rhodotorula* sp.	Rhosp1	1	Saprotroph
Fungi	Ascomycota	Dothideomycetes	Patellariales	Patellariaceae	*Rhytidhysteron*	*Rhytidhysteron rufulum*	Rhyru1	1	Saprotroph
Fungi	Basidiomycota	Agaricomycetes	Hymenochaetales	Repetobasidiaceae	*Rickenella*	*Rickenella mellea*	Ricme1	1	Pathogen
Fungi	Cryptomycota	Incertae sedis	Incertae sedis	Incertae sedis	*Rozella*	*Rozella allomycis*	Rozal1	2	Mycoparasite
Fungi	Ascomycota	Dothideomycetes	Botryosphaeriales	Botryosphaeriaceae	*Saccharata*	*Saccharata proteae*	Sacpr1	1	Pathogen
Fungi	Ascomycota	Saccharomycetes	Saccharomycetales	Saccharomycetaceae	*Saccharomyces*	*Saccharomyces cerevisiae*	Sacce1	1	Saprotroph
Fungi	Ascomycota	Saccharomycetes	Saccharomycetales	Saccharomycetaceae	*Saccharomyces*	*Saccharomyces cerevisiae*	SacceA	1	Saprotroph
Fungi	Ascomycota	Saccharomycetes	Saccharomycetales	Saccharomycetaceae	*Saccharomyces*	*Saccharomyces cerevisiae*	SacceB	1	Saprotroph
Fungi	Ascomycota	Saccharomycetes	Saccharomycetales	Saccharomycetaceae	*Saccharomyces*	*Saccharomyces cerevisiae*	SacceC	1	Saprotroph
Fungi	Ascomycota	Saccharomycetes	Saccharomycetales	Saccharomycetaceae	*Saccharomyces*	*Saccharomyces cerevisiae*	SacceD	1	Saprotroph
Fungi	Ascomycota	Saccharomycetes	Saccharomycetales	Saccharomycetaceae	*Saccharomyces*	*Saccharomyces cerevisiae*	SacceE	1	Saprotroph
Fungi	Ascomycota	Saccharomycetes	Saccharomycetales	Saccharomycetaceae	*Saccharomyces*	*Saccharomyces cerevisiae*	SacceY	1	Saprotroph
Fungi	Ascomycota	Taphrinomycetes	Taphrinales	Protomycetaceae	*Saitoella*	*Saitoella complicata*	Saico1	1	Saprotroph
Fungi	Basidiomycota	Agaricomycetes	Agaricales	Schizophyllaceae	*Schizophyllum*	*Schizophyllum commune*	Schco3	1	Saprotroph
Fungi	Basidiomycota	Agaricomycetes	Agaricales	Schizophyllaceae	*Schizophyllum*	*Schizophyllum commune*	SchcoA	1	Saprotroph
Fungi	Basidiomycota	Agaricomycetes	Agaricales	Schizophyllaceae	*Schizophyllum*	*Schizophyllum commune*	SchcoB	1	Saprotroph
Fungi	Basidiomycota	Agaricomycetes	Hymenochaetales	Schizoporaceae	*Schizopora*	*Schizopora paradoxa*	Schpa1	1	Saprotroph
Fungi	Ascomycota	Schizosaccharomycetes	Schizosaccharomycetales	Schizosaccharomycetaceae	*Schizosaccharomyces*	*Schizosaccharomyces cryophilus*	Schcy1	1	Saprotroph
Fungi	Ascomycota	Schizosaccharomycetes	Schizosaccharomycetales	Schizosaccharomycetaceae	*Schizosaccharomyces*	*Schizosaccharomyces japonicus*	Schja1	1	Saprotroph
Fungi	Ascomycota	Schizosaccharomycetes	Schizosaccharomycetales	Schizosaccharomycetaceae	*Schizosaccharomyces*	*Schizosaccharomyces octosporus*	Schoc1	1	Saprotroph
Fungi	Ascomycota	Schizosaccharomycetes	Schizosaccharomycetales	Schizosaccharomycetaceae	*Schizosaccharomyces*	*Schizosaccharomyces pombe*	Schpo1	1	Saprotroph
Fungi	Basidiomycota	Agaricomycetes	Boletales	Sclerodermataceae	*Scleroderma*	*Scleroderma citrinum*	Sclci1	1	Ectomycorrhizal
Fungi	Ascomycota	Leotiomycetes	Helotiales	Sclerotiniaceae	*Sclerotinia*	*Sclerotinia sclerotiorum*	Sclsc1	1	Pathogen
Fungi	Basidiomycota	Agaricomycetes	Sebacinales	Sebacinaceae	*Sebacina*	*Sebacina vermifera*	Sebve1	1	Ectomycorrhizal
Fungi	Ascomycota	Dothideomycetes	Capnodiales	Mycosphaerellaceae	*Septoria*	*Septoria musiva*	Sepmu1	1	Pathogen
Fungi	Ascomycota	Dothideomycetes	Capnodiales	Mycosphaerellaceae	*Septoria*	*Septoria populicola*	Seppo1	1	Pathogen
Fungi	Basidiomycota	Agaricomycetes	Boletales	Serpulaceae	*Serpula*	*Serpula lacrymans*	Serla_	1	Saprotroph
Fungi	Basidiomycota	Agaricomycetes	Boletales	Serpulaceae	*Serpula*	*Serpula lacrymans*	SerlaA	1	Saprotroph
Fungi	Basidiomycota	Agaricomycetes	Boletales	Serpulaceae	*Serpula*	*Serpula lacrymans*	SerlaB	1	Saprotroph
Fungi	Ascomycota	Dothideomycetes	Pleosporales	Pleosporaceae	*Setosphaeria*	*Setosphaeria turcica*	Settu1	2	Pathogen
Fungi	Basidiomycota	Agaricomycetes	Trechisporales	Hydnodontaceae	*Sistotremastrum*	*Sistotremastrum niveocremeum*	Sisni1	1	Saprotroph
Fungi	Basidiomycota	Agaricomycetes	Trechisporales	Hydnodontaceae	*Sistotremastrum*	*Sistotremastrum suecicum*	Sissu1	1	Saprotroph
Fungi	Ascomycota	Sordariomycetes	Hypocreales	Plectosphaerellaceae	*Sodiomyces*	*Sodiomyces alkalinus*	Sodal1	1	
Fungi	Ascomycota	Saccharomycetes	Saccharomycetales	Incertae sedis	*Spathaspora*	*Spathaspora passalidarum*	Spapa3	1	
Fungi	Ascomycota	Leotiomycetes	Rhytismatales	Cudoniaceae	*Spathularia*	*Spathularia flavida*	Spafl1	1	Saprotroph
Fungi	Basidiomycota	Agaricomycetes	Geastrales	Geastraceae	*Sphaerobolus*	*Sphaerobolus stellatus*	Sphst1	1	Saprotroph
Fungi	Basidiomycota	Ustilaginomycetes	Ustilaginales	Ustilaginaceae	*Sporisorium*	*Sporisorium reilianum*	Spore1	1	Pathogen
Fungi	Basidiomycota	Microbotryomycetes	Sporidiobolales	Incertae sedis	*Sporobolomyces*	*Sporobolomyces linderae*	Spoli1	1	Mycoparasite
Fungi	Basidiomycota	Microbotryomycetes	Sporidiobolales	Incertae sedis	*Sporobolomyces*	*Sporobolomyces roseus*	Sporo1	1	Mycoparasite
Fungi	Ascomycota	Dothideomycetes	Pleosporales	Sporormiaceae	*Sporormia*	*Sporormia fimetaria*	Spofi1	2	Saprotroph
Fungi	Ascomycota	Dothideomycetes	Pleosporales	Phaeosphaeriaceae	*Stagonospora*	*Stagonospora nodorum*	Stano2	2	Pathogen
Fungi	Ascomycota	Dothideomycetes	Pleosporales	Phaeosphaeriaceae	*Stagonospora*	*Stagonospora* sp.	Stasp1	1	Pathogen
Fungi	Ascomycota	Sordariomycetes	Hypocreales	Bionectriaceae	*Stanjemonium*	*Stanjemonium grisellum*	Stagr1	1	Saprotroph
Fungi	Basidiomycota	Agaricomycetes	Russulales	Stereaceae	*Stereum*	*Stereum hirsutum*	Stehi1	1	Saprotroph
Fungi	Basidiomycota	Agaricomycetes	Boletales	Suillaceae	*Suillus*	*Suillus brevipes*	Suibr1	1	Ectomycorrhizal
Fungi	Basidiomycota	Agaricomycetes	Boletales	Suillaceae	*Suillus*	*Suillus luteus*	Suilu1	1	Ectomycorrhizal
Fungi	Ascomycota	Incertae sedis	Incertae sedis	Incertae sedis	*Symbiotaphrina*	*Symbiotaphrina kochii*	Symko1	1	Animal endosymbiont
Fungi	Ascomycota	Eurotiomycetes	Eurotiales	Trichocomaceae	*Talaromyces*	*Talaromyces aculeatus*	Penac1	1	Saprotroph
Fungi	Ascomycota	Eurotiomycetes	Eurotiales	Trichocomaceae	*Talaromyces*	*Talaromyces marneffei*	Talma1	1	Saprotroph
Fungi	Ascomycota	Eurotiomycetes	Eurotiales	Trichocomaceae	*Talaromyces*	*Talaromyces stipitatus*	Talst1	1	Saprotroph
Fungi	Ascomycota	Taphrinomycetes	Taphrinales	Taphrinaceae	*Taphrina*	*Taphrina deformans*	Tapde1	2	Pathogen
Fungi	Ascomycota	Dothideomycetes	Capnodiales	Teratosphaeriaceae	*Teratosphaeria*	*Teratosphaeria nubilosa*	Ternu1	1	Pathogen
Fungi	Ascomycota	Pezizomycetes	Pezizales	Pezizaceae	*Terfezia*	*Terfezia boudieri*	Terbo1	1	Ectomycorrhizal
Fungi	Ascomycota	Pezizomycetes	Pezizales	Pezizaceae	*Terfezia*	*Terfezia boudieri*	Terbo2	1	Ectomycorrhizal
Fungi	Basidiomycota	Ustilaginomycetes	Ustilaginales	Anthracoideaceae	*Testicularia*	*Testicularia cyperi*	Tescy1	1	Pathogen
Fungi	Ascomycota	Leotiomycetes	Thelebolales	Thelebolaceae	*Thelebolus*	*Thelebolus microsporus*	Themi1	1	Saprotroph

Continuation **Table S4**

Kingdom	Phylum	Class	Order	Family	Genus	Species	JGI nomenclature	Number of copies	Ecology
Fungi	Ascomycota	Leotiomycetes	Thelebolales	Thelebolaceae	*Thelebolus*	*Thelebolus stercoreus*	Thest1	1	Saprotroph
Fungi	Ascomycota	Eurotiomycetes	Eurotiales	Thermoascaceae	*Thermoascus*	*Thermoascus aurantiacus*	Theau1	1	Saprotroph
Fungi	Ascomycota	Sordariomycetes	Sordariales	Chaetomiaceae	*Thielavia*	*Thielavia antarctica*	Thian1	1	Saprotroph
Fungi	Ascomycota	Sordariomycetes	Sordariales	Chaetomiaceae	*Thielavia*	*Thielavia appendiculata*	Thiap1	1	Saprotroph
Fungi	Ascomycota	Sordariomycetes	Sordariales	Chaetomiaceae	*Thielavia*	*Thielavia arenaria*	Thiar1	2	Saprotroph
Fungi	Ascomycota	Sordariomycetes	Sordariales	Chaetomiaceae	*Thielavia*	*Thielavia hyrcaniae*	Thihy1	1	Saprotroph
Fungi	Ascomycota	Sordariomycetes	Sordariales	Chaetomiaceae	*Thielavia*	*Thielavia terrestris*	Thite2	2	Saprotroph
Fungi	Ascomycota	Sordariomycetes	Chaetosphaeriales	Chaetosphaeriaceae	*Thozetella*	*Thozetella sp.*	ThoPMI	4	Saprotroph
Fungi	Basidiomycota	Exobasidiomycetes	Georgefischeriales	Tilletiariaceae	*Tilletiaria*	*Tilletiaria anomala*	Tilan2	1	Pathogen
Fungi	Basidiomycota	Exobasidiomycetes	Incertae sedis	Incertae sedis	*Tilletiopsis*	*Tilletiopsis washingtonensis*	Tilwa1	1	
Fungi	Ascomycota	Saccharomycetes	Saccharomycetales	Saccharomycetaceae	*Torulaspora*	*Torulaspora delbrueckii*	Torde1	1	Saprotroph
Fungi	Ascomycota	Dothideomycetes	Microthyriales	Microthyriaceae	*Tothia*	*Tothia fuscella*	Totfu1	2	Saprotroph
Fungi	Basidiomycota	Agaricomycetes	Polyporales	Polyporaceae	*Trametes*	*Trametes ljubarskyi*	Tralj1	1	Saprotroph
Fungi	Basidiomycota	Agaricomycetes	Polyporales	Polyporaceae	*Trametes*	*Trametes versicolor*	Trave1	1	Saprotroph
Fungi	Ascomycota	Dothideomycetes	Pleosporales	Pleomassariaceae	*Trematosphaeria*	*Trematosphaeria pertusa*	Trepe1	1	Saprotroph
Fungi	Basidiomycota	Tremellomycetes	Tremellales	Incertae sedis	*Tremella*	*Tremella mesenterica*	Treme1	0	Mycoparasite
Fungi	Basidiomycota	Agaricomycetes	Hymenochaetales	Incertae sedis	*Trichaptum*	*Trichaptum abietinum*	Triab1	1	Saprotroph
Fungi	Ascomycota	Dothideomycetes	Pleosporales	Phaeotrichaceae	*Trichodelitschia*	*Trichodelitschia bisporula*	Tribi1	1	Saprotroph
Fungi	Ascomycota	Sordariomycetes	Hypocreales	Hypocreaceae	*Trichoderma*	*Trichoderma asperellum*	Trias1	1	Saprotroph
Fungi	Ascomycota	Sordariomycetes	Hypocreales	Hypocreaceae	*Trichoderma*	*Trichoderma atroviride*	Triat2	1	Saprotroph
Fungi	Ascomycota	Sordariomycetes	Hypocreales	Hypocreaceae	*Trichoderma*	*Trichoderma harzianum*	Triha1	1	Saprotroph
Fungi	Ascomycota	Sordariomycetes	Hypocreales	Hypocreaceae	*Trichoderma*	*Trichoderma longibrachiatum*	Trilo3	1	Saprotroph
Fungi	Ascomycota	Sordariomycetes	Hypocreales	Hypocreaceae	*Trichoderma*	*Trichoderma reesei*	Trire2	1	Saprotroph
Fungi	Ascomycota	Sordariomycetes	Hypocreales	Hypocreaceae	*Trichoderma*	*Trichoderma reesei*	TrireR	1	Saprotroph
Fungi	Ascomycota	Sordariomycetes	Hypocreales	Hypocreaceae	*Trichoderma*	*Trichoderma virens*	TriviG	1	Saprotroph
Fungi	Basidiomycota	Agaricomycetes	Agaricales	Tricholomataceae	*Tricholoma*	*Tricholoma matsutake*	Trima3	1	Ectomycorrhizal
Fungi	Ascomycota	Saccharomycetes	Saccharomycetales	Trichomonascaceae	*Trichomonascus*	*Trichomonascus petasosporus*	Tripe1	1	Saprotroph
Fungi	Ascomycota	Eurotiomycetes	Onygenales	Arthrodermataceae	*Trichophyton*	*Trichophyton rubrum*	Triru1	1	Saprotroph
Fungi	Ascomycota	Eurotiomycetes	Onygenales	Arthrodermataceae	*Trichophyton*	*Trichophyton verrucosum*	Triver	1	Saprotroph
Fungi	Basidiomycota	Tremellomycetes	Trichosporonales	Trichosporonaceae	*Trichosporon*	*Trichosporon chiarellii*	Trich1	0	Animal parasite
Fungi	Basidiomycota	Tremellomycetes	Trichosporonales	Trichosporonaceae	*Trichosporon*	*Trichosporon oleaginosus*	Triol1	0	Animal parasite
Fungi	Ascomycota	Dothideomycetes	Pleosporales	Incertae sedis	*Trinosporium*	*Trinosporium guianense*	Trigu1	1	
Fungi	Basidiomycota	Tritirachiomycetes	Incertae sedis	Incertae sedis	*Tritirachium*	*Tritirachium* sp.	Trisp1	0	Saprotroph
Fungi	Ascomycota	Dothideomycetes	Trypetheliales	Trypetheliaceae	*Trypethelium*	*Trypethelium eluteriae*	Tryvi1	1	Lichenized
Fungi	Ascomycota	Pezizomycetes	Pezizales	Tuberaceae	*Tuber*	*Tuber melanosporum*	Tubme1	1	Ectomycorrhizal
Fungi	Basidiomycota	Agaricomycetes	Cantharellales	Tulasnellaceae	*Tulasnella*	*Tulasnella calospora*	Tulca1	1	Saprotroph
Fungi	Zygomycota	Incertae sedis	Mucorales	Umbelopsidaceae	*Umbelopsis*	*Umbelopsis ramanniana*	Umbra1	1	Saprotroph
Fungi	Ascomycota	Eurotiomycetes	Onygenales	Onygenaceae	*Uncinocarpus*	*Uncinocarpus reesii*	Uncre1	1	Saprotroph
Fungi	Basidiomycota					*Ustilaginomycotina* sp.	Ustsp1	1	
Fungi	Basidiomycota	Ustilaginomycetes	Ustilaginales	Ustilaginaceae	*Ustilago*	*Ustilago maydis*	Ustma1	1	Pathogen
Fungi	Ascomycota	Sordariomycetes	Hypocreales	Niessliaceae	*Valetoniellopsis*	*Valetoniellopsis laxa*	Valla1	1	Saprotroph
Fungi	Ascomycota	Dothideomycetes	Pleosporales	Venturiaceae	*Venturia*	*Venturia inaequalis*	Venin1	2	Pathogen
Fungi	Ascomycota	Dothideomycetes	Pleosporales	Testudinaceae	*Verruculina*	*Verruculina enalia*	Veren1	2	Saprotroph
Fungi	Ascomycota	Sordariomycetes	Hypocreales	Plectosphaerellaceae	*Verticillium*	*Verticillium alfalfae*	Veral1	1	Pathogen
Fungi	Ascomycota	Sordariomycetes	Hypocreales	Plectosphaerellaceae	*Verticillium*	*Verticillium dahliae*	Verda1	1	Pathogen
Fungi	Basidiomycota	Agaricomycetes	Agaricales	Pluteaceae	*Volvariella*	*Volvariella volvacea*	Volvo1	1	Saprotroph
Fungi	Basidiomycota	Wallemiomycetes	Wallemiales	Wallemiaceae	*Wallemia*	*Wallemia ichthyophaga*	Walic1	0	Saprotroph
Fungi	Basidiomycota	Wallemiomycetes	Wallemiales	Wallemiaceae	*Wallemia*	*Wallemia sebi*	Walse1	0	Saprotroph
Fungi	Ascomycota	Dothideomycetes	Pleosporales	Sporormiaceae	*Westerdykella*	*Westerdykella ornata*	Wesor1	2	Saprotroph
Fungi	Ascomycota	Saccharomycetes	Saccharomycetales	Incertae sedis	*Wickerhamomyces*	*Wickerhamomyces anomalus*	Wican1	1	
Fungi	Ascomycota	Pezizomycetes	Pezizales	Pyronemataceae	*Wilcoxina*	*Wilcoxina mikolae*	Wilmi1	1	Ectomycorrhizal
Fungi	Basidiomycota	Agaricomycetes	Polyporales	Polyporaceae	*Wolfiporia*	*Wolfiporia cocos*	Wolco1	1	Saprotroph
Fungi	Ascomycota	Lecanoromycetes	Teloschistales	Teloschistaceae	*Xanthoria*	*Xanthoria parietina*	Xanpa1	1	Lichenized
Fungi	Ascomycota	Lecanoromycetes	Teloschistales	Teloschistaceae	*Xanthoria*	*Xanthoria parietina*	Xanpa2	1	Lichenized
Fungi	Ascomycota	Xylonomycetes	Xylonomycetales	Xylonomycetaceae	*Xylona*	*Xylona heveae*	Xylhe1	1	Endophyte
Fungi	Ascomycota	Saccharomycetes	Saccharomycetales	Incertae sedis	*Yarrowia*	*Yarrowia lipolytica*	Yarli1	1	
Fungi	Ascomycota	Dothideomycetes	Capnodiales	Mycosphaerellaceae	*Zasmidium*	*Zasmidium cellare*	Zasce1	2	
Fungi	Ascomycota	Dothideomycetes	Pleosporales	Zopfiaceae	*Zopfia*	*Zopfia rhizophila*	Zoprh1	2	Saprotroph
Fungi	Ascomycota	Saccharomycetes	Saccharomycetales	Saccharomycetaceae	*Zygosaccharomyces*	*Zygosaccharomyces rouxii*	Zygro1	1	Saprotroph
Fungi	Ascomycota	Incertae sedis	Incertae sedis	Incertae sedis	*Zymoseptoria*	*Zymoseptoria ardabiliae*	Zymar1	1	
Fungi	Ascomycota	Incertae sedis	Incertae sedis	Incertae sedis	*Zymoseptoria*	*Zymoseptoria pseudotritici*	Zymps1	1	
Chromista	Oomycota	Oomycetes	Peronosporales	Phythiaceae	*Phytophtora*	*Phytophtora capsici*		1	Pathogen
Chromista	Oomycota	Oomycetes	Peronosporales	Phythiaceae	*Phytophtora*	*Phytophtora cinnamomi*		1	Pathogen
Chromista	Oomycota	Oomycetes	Peronosporales	Phythiaceae	*Phytophtora*	*Phytophtora sojae*	Physo1_1	1	Pathogen
Chromista	Oomycota	Oomycetes	Peronosporales	Phythiaceae	*Phytophtora*	*Phytophtora ramorum*	Phyra1_1	1	Pathogen

GENERAL CONCLUSIONS

CONCLUSIONS

1.- The tree species genotype and identity, together with seasonal variations and ecosystem development, i.e., fire regime, determine the overall quality of soils, as well as processes related to carbon and nutrient cycling in Mediterranean pine forests.

2.- Seasonal local and regional diversity patterns of soil fungal communities associated with *Pinus pinaster* vary depending on the functional and/or taxonomic fungal guild, with richer and more heterogeneous ectomycorrhizal communities in spring than autumn, and saprotrophic, ascomycetous and zygomycetous communities in autumn.

3.- Indicator soil fungi are found beneath distinct *Pinus pinaster* genotypes, and host preferences are betimes context dependent, indicating host and environmental filtering as important mechanisms structuring soil fungal communities in these forests.

4.- High productive Mediterranean tree genotypes generate different soil quality and associate different ectomycorrhizal assemblages where Basidiomycetes prevail, while less productive Atlantic trees preferentially associate Ascomycetes fungi.

5.- The tree genotype together with the local environmental conditions explain the phylogenetic assemblage of rhizospheric ectomycorrhizal and bacterial communities, as well as of fungal communities in the bulk soil.

6.- Distinct *Pinus pinaster* genotypes modified ecosystem services related to the cycling of nutrients by direct variation of the soil quality, and through modulating the phylogenetic structure of soil fungal communities. Concrete ecosystem processes were identified associated with specific phylogenetic clades inside the main ecological fungal guilds in forest soils, i.e., ectomycorrhizal and saprotrophic.

7.- In all cases, the structural adjustments of fungal and bacterial communities have functional consequences on nutrient cycling processes. Mechanisms of functional complementarity are proposed since fungi and bacteria explain the activity of different C and N enzymes, similarly to that observed for rhizospheric and soil fungi and C, N and P cycling enzymes.

8.- Natural populations of *Pinus pinaster* and *Pinus halepensis* present contrasted edaphic environments, as well as distinct species composition within their associated fungal communities.

9.- The local and regional diversity of ectomycorrhizal fungi in the rhizosphere of *Pinus pinaster* and *Pinus halepensis* is reduced in serotinous populations located in areas of high fire recurrence.

10.- Both pine species harbor similarly enriched ECM fungal communities in roots, but different species assemblages, which also diverge in their functional response to the fire regime. Main

functional adjustments derived from structural shifts mediated by high fire recurrence and/or serotiny are linked to increased carbon turnover and reduced mobilization of nitrogen.

11.- The genetics of Mediterranean pine populations subjected to contrasted fire regimes regulates the phylogenetic structure of their associated fungal communities in soil, but not in root-tips.

12.- The structural and functional response of fungal communities of *Pinus pinaster* and *Pinus halepensis* forests is different depending on the edaphic compartment, indicating the relevance of spatial partitioning and niche differentiation processes, especially evidenced for ectomycorrhizal fungi.

13.- Contrarily to that observed for local α-diversity, the fire regime does not affect the phylogenetic structure of root-tip fungal communities, but it prints a phylogenetic signal on those in the bulk soil, particularly in the case of *Pinus halepensis*.

14.- The high fire recurrence drives the phylogenetic clustering of soil fungal communities in *Pinus halepensis* forests, further explained by the over-representation of Basidiomycetes. These fire regime effects are explained through changes induced in the soil quality along the ecosystem development.

15.- Variations in the phylogenetic structure of root-tip and bulk soil fungal communities under different fire regimes entail functional consequences related to the cycling of nutrients in *Pinus pinaster* and *Pinus halepensis* forests. Concrete ecosystem processes were identified associated with specific phylogenetic fungal clades. In particular, the prevalence of Basidiomycetes in soils of high recurrently burned *Pinus halepensis* forests significantly enhanced the mobilization of phosphorous and nitrogen.

16.- Because the tree genotype, the season, and the fire regime left a phylogenetic signature in the structure of microbial communities that further had implications on the ecosystem functioning, phylogenetic approaches emerge as a highly valuable tool to better understanding the relation among microbial diversity and ecosystem functioning.

17.- Glycosyl Hydrolase family 63 is proposed as a new molecular marker indicator of the structure and diversity of fungal communities with barcoding and phylogenetic abilities, and with potential to be an indicator of the secretory machinery of these communities. The primers of fungal GH63 validated in our study provide a valuable tool in environmental metagenomics studies to link community composition to fungal functions.

CONCLUSIONES GENERALES

1.- Tanto el genotipo como la identidad de las especies arbóreas, junto con las variaciones estacionales y el régimen de incendios, determinan la calidad general de los suelos, así como los procesos relacionados con los ciclos de carbono y nutrientes en los bosques de pino Mediterráneo.

2.- Los patrones estacionales de diversidad local y regional de las comunidades fúngicas del suelo asociadas con *Pinus pinaster* varían dependiendo del consorcio fúngico funcional y/o taxonómico, con comunidades ectomicorrícicas más ricas y heterogéneas en primavera, y comunidades de saprófitos, ascomicetes y zigomicetes más ricas en otoño.

3.- Los distintos genotipos de *Pinus pinaster* seleccionan hongos indicadores, y las preferencias por el huésped son dependientes del las condiciones locales, lo que indica que el huésped y filtro ambiental son mecanismos importantes para estructurar las comunidades fúngicas del suelo en estos bosques.

4.- Los genotipos de árboles Mediterráneos, con alta productividad, generan diferente calidad de suelo y asocian diferentes consorcios ectomicorrícicos donde prevalecen los hongos basidiomicetes, mientras que los árboles Atlánticos, menos productivos, asocian preferentemente hongos ascomicetes.

5.- El genotipo del árbol junto con las condiciones ambientales locales explican el ensamblaje filogenético de las comunidades ectomicorrícicas y bacterianas de la rizosfera, así como de las comunidades fúngicas de la matriz del suelo.

6.- Distintos genotipos de *Pinus pinaster* modifican los servicios ecosistémicos relacionados con el ciclado de nutrientes mediante variaciones directas de la calidad del suelo y modulando la estructura filogenética de las comunidades de hongos del suelo. Se han identificado procesos ecosistémicos concretos asociados con clados filogenéticos específicos dentro de los principales consorcios funcionales de hongos en suelos forestales, es decir, hongos ectomicorrícicos y saprófitos.

7.- En todos los casos, los ajustes estructurales de las comunidades fúngicas y bacterianas tienen consecuencias funcionales sobre los procesos del ciclado de nutrientes. Se proponen mecanismos de complementariedad funcional, ya que los hongos y las bacterias explican la actividad de diferentes enzimas relacionadas con los ciclos del C y N, de forma similar a la observada para los hongos rizosféricos y del suelo y las enzimas del C, N y P.

8.- Las poblaciones naturales de *Pinus pinaster* y *Pinus halepensis* presentan ambientes edáficos contrastados, así como una composición distinta de especies dentro de sus comunidades de hongos asociadas.

9.- La diversidad local y regional de los hongos ectomicorrícicos en la rizosfera de *Pinus pinaster* y *Pinus halepensis* disminuye en poblaciones serótinas localizadas en áreas de alta recurrencia de incendios.

10.- Ambas especies de pino albergan comunidades fúngicas ECM en las raíces similares en riqueza, pero diferentes en cuanto a composición de especies, que a su vez difieren en su respuesta funcional al régimen de incendios. Los principales ajustes funcionales derivados de los cambios estructurales mediados por la alta recurrencia de incendios y/o la serotinia están relacionados con el aumento del ciclado del carbono y la reducción de la movilización de nitrógeno.

11.- La genética de las poblaciones de pino Mediterráneo sometidas a regímenes de incendios contrastados regula la estructura filogenética de las comunidades fúngicas asociadas en el suelo, pero no en las raíces cortas.

12.- La respuesta estructural y funcional de las comunidades fúngicas de los bosques de *Pinus pinaster* y *Pinus halepensis* es diferente dependiendo del compartimiento edáfico, lo que pone de manifiesto la relevancia de los procesos de partición espacial y diferenciación de nicho, especialmente evidenciados para hongos ectomicorrícicos

13.- Contrariamente a lo observado para la diversidad local, el régimen de incendios no afecta a la estructura filogenética de las comunidades fúngicas de las raíces cortas, pero imprime una señal filogenética en las comunidades de la matriz del suelo, particularmente en el caso de *Pinus halepensis.*

14.- La alta recurrencia de incendios impulsa el agrupamiento filogenético de las comunidades fúngicas del suelo en los bosques de *Pinus halepensis*, explicado además por la sobrerrepresentación de Basidiomicetes. Estos efectos del régimen de incendios se explican a través de cambios inducidos en la calidad del suelo a lo largo del desarrollo del ecosistema.

15.- Las variaciones en la estructura filogenética de las comunidades fúngicas de las raíces y del suelo en diferentes regímenes de incendios implican consecuencias funcionales relacionadas con el ciclado de nutrientes en los bosques de *Pinus pinaster* y *Pinus halepensis*. Se han identificado procesos ecosistémicos concretos asociados con clados filogenéticos fúngicos específicos. En particular, la prevalencia de basidiomicetes en suelos recurrentemente quemados de *Pinus halepensis* mejora significativamente la movilización de fósforo y nitrógeno.

16.- Debido a que el genotipo del árbol, la estación y el régimen de incendios dejan una huella filogenética en la estructura de las comunidades microbianas que además tene implicaciones en el funcionamiento del ecosistema, los enfoques filogenéticos emergen como una herramienta muy

valiosa para comprender mejor las relaciones entre la diversidad microbiana y el funcionamiento del ecosistema.

17.- La familia Glicosil Hidrolasa 63 se propone como un nuevo marcador molecular indicador de la estructura y diversidad de comunidades fúngicas, con capacidades filogenéticas y de identificación taxonómica y con potencial para ser un indicador de la maquinaria secretora de estas comunidades. Los cebadores de hongos GH63 validados en nuestro estudio proporcionan una herramienta muy valiosa para estudios de metagenómica con muestras ambientales cuyo objetivo sea vincular funcionalidad con la composición de la comunidad fúngica.

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